Genetic relationship of wood and plains bison based on restriction fragment length polymorphisms

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Received August 21, 1989

BORK, A. M., STROBECK, C. M., YEH, F. C., HUDSON, R. J., and SALMON, R. K. 1991. Genetic relationship of wood and plains bison based on restriction fragment length polymorphisms. Can. J. Zool. 69: 43–48.

To evaluate the genetic relationship within and between wood and plains bison of Elk Island National Park, genomic DNA samples were screened for restriction fragment length polymorphisms (RFLP) with cDNA probes for growth hormone, growth hormone releasing factor, somatostatin, and insulin-like growth factor-1. Of the 28 fragments identified, two revealed RFLPs, both of which were associated with the growth hormone releasing factor locus. The observed frequencies of the polymorphic sites did not differ from a Hardy–Weinberg distribution in either population, which is indicative of random mating populations. The contingency χ^2 tests for homogeneity indicate that the fragment frequencies of the polymorphic restriction sites differ significantly ($p = 0.00$) between the wood and plains bison. The number of net nucleotide substitutions between the two populations was 0.0007, indicative of a recent divergence. Conversion of the bison nucleotide divergence results in a relative protein divergence of 0.007 to 0.018. This converted divergence corresponds closely to the divergence reported for other geographically isolated populations; thus, this preliminary analysis suggests the bison have at least reached the stage of geographic isolation in their evolutionary divergence.

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Nous avons évalué la relation génétique au sein des populations de bisons des bois et de bisons des plaines d'une part, et entre ces populations d'autre part, dans le parc national d'Elk Island; des sondes d'ADN complémentaire capables de repérer l'hormone de croissance, le facteur de libération de l'hormone de croissance, la somatostatine et le facteur-1 de croissance semblable à l'insuline ont servi à retrouver les polymorphismes des fragments de restriction (RFLP) dans des échantillons d'ADN génomique. Des 28 fragments identifiés, deux étaient le siège de RFLPs, tous deux associés au locus du facteur de libération de l'hormone de croissance. Les fréquences observées des sites de polymorphisme correspondaient à une distribution Hardy–Weinberg dans les deux populations, ce qui reflète un système où les accouplements se font au hasard. Les tests de contingence du χ^2, destinés à mesurer l'homogénéité, ont montré que la fréquence des fragments aux sites des restrictions polymorphes différait significativement ($P = 0{,}00$) chez les bisons des plaines et les bisons des bois. Le nombre de substitutions nettes de nucléotides entre les deux populations était de 0,0007, ce qui reflète une divergence récente. La conversion de cette divergence des nucléotides résulte en une divergence relative de protéines de 0,007 à 0,018. Cette divergence convertie correspond étroitement à la divergence constatée chez d'autres populations isolées géographiquement; cette analyse préliminaire indique donc que les deux types de bisons ont atteint au moins le stade d'isolement géographique dans leur divergence évolutive.

[Traduit par la revue]

Introduction

The existence of a distinct form of bison in northern Canada was recognized over 100 years ago and eventually culminated in the designation of this northern form as the subspecies *Bison bison athabascae*, commonly known as the wood bison (van Zyll de Jong 1986). However, the lack of an unambiguous concept of a subspecies has resulted in a persistent controversy concerning the validity of this subspecific designation as distinct from the plains bison (*Bison bison bison*) (Peden and Kraay 1979; McDonald 1981; van Zyll de Jong 1986). Wood bison are usually distinguished from plains bison by their larger body parameters, larger horn cores, dark woolly pelage, and a relatively small amount of hair on their upper forelegs and beard (Geist and Karsten 1977; Reynolds *et al.* 1982). Using multivariate morphometrics to analyze body size parameters, van Zyll de Jong (1986) considered that the phenotypic discontinuity in body size parameters fully justified the subspecific distinctions. However, the conclusion of van Zyll de Jong (1986) is inconsistent with the previous observations of Peden and Kraay (1979) who found that the blood types and carbonic anhydrase polymorphisms in wood, plains, and wood × plains hybrid bison were similar. On the basis of their study, Peden and Kraay (1979) argued that the classification of wood and plains bison as separate subspecies could be questioned. Other comparisons of the blood types and karyotypes of wood and plains bison (Ying and Peden 1977; Zamora 1983) have been no more successful in clarifying the taxonomic relationships between the two bison types.

Several recent studies have revealed the potential of restriction endonuclease analysis of mitochondrial DNA to clarify the genetic relationships and evolutionary histories of various groups of organisms (e.g., Avise *et al.* 1979*b*; Ferris *et al.* 1983*a*; Ferris *et al.* 1983*b*; Powell 1983; Wright *et al.* 1983; Spolsky and Uzzell 1986). However, preliminary work with

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wood and plains bison mitochondrial DNA has been inconclusive as additional comparisons between the subspecies are required to establish criteria for classification (Cronin 1986).

In a restriction site analysis of mice with divergent growth patterns, Salmon *et al.* (1988) reported that genetic variation at the growth hormone gene locus was strongly associated with body size. Since body size parameters are used to differentiate between wood and plains bison, variation may exist at growth-regulating genes within the two bison types. Therefore, the study reported herein employed restriction endonuclease analysis of four genes (growth hormone (GH), growth hormone releasing factor (GHRF), somatostatin (S), and insulin-like growth factor-1 (IGF-1)) known to regulate postnatal mammalian growth (Raisz and Kream 1981; Froesch *et al.* 1985; Zapf and Froesch 1986; Gorbman *et al.* 1983) to explore the genetic relationships between the two North American bison types.

Materials and methods

Sample collection

Approximately 10 mL of blood was drawn from the tail vein of 40 plains bison and 40 wood bison randomly selected from the two bison populations maintained in Elk Island National Park (EINP). The blood was collected in EDTA vacutainers (Beckton Dickinson and Company, Rutherford, NJ) and immediately stored between 0 and 4°C (wet ice or refrigerator) until DNA extraction.

Four European bison (wisent) samples were obtained for comparison with polymorphic loci discovered between the two North American bison subspecies. Three wisent DNA samples, which originated in Poland, were generously provided by M. Fellous (Institut Pasteur, France). The fourth wisent blood sample was provided by C. G. Penny (San Diego Zoo, CA).

DNA preparation

To facilitate the extraction of DNA, white blood cells were isolated and subsequently lysed. To isolate white blood cells, five volumes of a 0.155 M NH_4Cl – 0.17 M Tris solution (prewarmed to 37°C) was added to one volume of whole blood. Following a 5-min incubation at 37°C, the solution was centrifuged at 2000 rpm (Beckman JA-20 rotor) for 10 min. After aspiration of the supernatant, the pellet was resuspended in 10 mL of a 0.85% NaCl solution and centrifuged at 2000 rpm for 10 min. This sequence of aspiration, resuspension in 0.85% NaCl, centrifugation, and aspiration was then repeated. The white blood cells in the final pellet were lysed by resuspending this pellet in 2 mL of 100 mM Tris (pH 8.0) containing 1 mM EDTA and then immediately injecting 2 mL of 100 mM Tris (pH 8.0) containing 40 mM EDTA and 1.2% SDS.

For the extraction of DNA, an equal volume of phenol saturated with TE (10 mM Tris-HCl and 1 mM EDTA, pH 8.0) was added to the solution of lysed cells and mixed for 10 min. The resulting emulsion was then centrifuged at 5000 rpm (Beckman JA-20 rotor) for 5 min. After centrifugation, the upper aqueous phase was reextracted with an equal volume of TE-saturated phenol. This second phenol extraction was followed by an extraction with an equal volume of 1:1 phenol–chloroform and, subsequently, with an equal volume of 24:1 chloroform – isoamyl alcohol. The final aqueous phase was then dialysed against three changes of TE over a 24-h period. Following dialysis, DNA was ethanol precipitated and resuspended in TE.

A total of 10 commercially obtained restriction endonucleases (Bethesda Research Laboratories, Burlington, Ont.; Pharmacia, Dorval, Que.) were used to digest the bison DNAs. Addition of the restriction enzyme was carried out over 6 h and total digestion time ranged between 18 and 22 h. The digested DNAs were electrophoresed on 0.7% agarose gels in 0.04 M Tris-acetate buffer containing 1 mM EDTA (Maniatis *et al.* 1982) at 30 V for 20–24 h. Ethidium bromide (0.3 μg/mL) was added to the gel to allow visualization of DNA. Each gel included DNA markers of known fragment sizes: bacteriophage λ [illegible] 22 cleaved with *Sau*3AI, as well as high molecular weight (HMW) marker (BRL). The size of the marker fragments ranged from 48 to 0.36 kb to allow for an estimation of bison fragment size. After electrophoresis, the digested DNA fragments were transferred onto a nylon membrane (GeneScreen Plus, New England Nuclear Research Products, Boston, MA). Treatment of the DNA before and after transfer to the membrane followed the conditions recommended by the supplier. The method of transfer followed the protocol outlined in Maniatis *et al.* (1982). Transfer was carried out for 38–48 h.

The four cDNAs (complementary DNAs) used for hybridization analysis were bovine growth hormone (bGH/pSP65) (Gordon *et al.* 1983), human somatostatin (pHS/pRTB1-63) (Shen *et al.* 1982), human insulin-like growth factor-I (hIGF-1B/pGEM1) (Rotwein 1986), and rat growth hormone releasing factor (hGRF/λ101) (Mayo *et al.* 1985). Plasmids containing the above cDNA were transformed into appropriate host bacterial strains, isolated, and purified following the procedures outlined in Maniatis *et al.* (1982). Inserts were cleaved from plasmids by restriction enzyme digestion and separated from vector DNA and LMP agarose (BRL) gels. Probes were radiolabelled with [^{32}P]dCTP (ICN) by the random primer method (Feinberg and Vogelstein 1983, 1984). The labelled probe fragments were separated from the unincorporated radionucleotides by spun-column chromatography (Maniatis *et al.* 1982). Prehydridization, hybridization, and washing of the membrane followed the formamide procedure recommended by the supplier. Membranes were autoradiographed at −70°C using two sheets of Kodak GBX-2 film (Eastman Kodak Co., Rochester, NY) and Lightning Plus intensifying screens (Dupont). Films were exposed for 3–14 days depending on probe activity.

Statistical analysis

The fragments, identified via autoradiography, were considered in terms of restriction site locality. Although more complex sequence alterations could undoubtedly occur, the assumption was made that a dimorphic restriction fragment reflected the existence of a single dimorphic restriction site.

The genotypic frequencies for the wood and plains bison populations were calculated for each polymorphic site identified and were compared to Hardy–Weinberg proportions. The differences between the observed and expected values were analyzed using χ^2 tests corrected for small sample size (Levene 1949). The extent of homogeneity of fragment frequencies for each polymorphic site between the bison populations was estimated through the contingency χ^2 analysis (Feinberg 1983).

DNA divergence between the two subspecies was determined through Nei's (1987) formula. The number of net nucleotide substitutions (DNA divergence) between two populations (D) is estimated by

$$[1] \quad D = D_{xy} - (D_x + D_y)/2$$

where D_x is the average number of nucleotide substitutions for DNA haplotypes in population x, D_y is the average number of nucleotide substitutions for haplotypes in population y, and D_{xy} is the average number of nucleotide substitutions between DNA haplotypes from populations x and y. D_x, D_y, and D_{xy} were calculated using equation 5.42 (Nei 1987, p. 101) and equations 10.19 and 10.20 (Nei 1987, p. 276). The haplotype frequencies used for this calculations are given in Table 1.

Results

The 22 enzyme–probe combinations used in this study provided 28 fragments (50 restriction sites) for the examination of intra-and inter-population genetic variability (see Table 2). Of these 28 fragments, two revealed restriction fragment length polymorphisms (RFLPs), both of which were associated with the GHRF locus. When digested with *Eco*RV, the bison GHRF locus yielded fragments that were either 8.9 or 14.4 kb; *Sph*I digestion of this locus yielded fragments that were either 11.1 or 12.6 kb in length (Fig. 1). Each of these RFLPs was identified within both the wood and plains bison samples. These fragments were interpreted as diallelic polymorphisms at each of

TABLE 1. Observed genotypes at *Sph*I and *Eco*RV polymorphic sites

	*Sph*I		
*Eco*RV	S_1S_1	S_1S_2	S_2S_2
E_1E_1	10 wood	0	0
E_1E_2	4 wood 3 plains	6 wood 5 plains	0
E_2E_2	1 plains	7 plains	4 plains

NOTE: Frequencies of haplotypes: $E_1S_1 = 38$, $E_1S_2 = 0$, $E_2S_1 = 16$, and $E_2S_2 = 26$. Since no single or double homozygotes involving E_1S_2 haplotype were observed, it is assumed the "double heterozygotes" consist of haplotypes E_1S_1 and E_2S_2.

TABLE 2. Restriction fragment lengths associated with growth-regulating loci

Enzyme	Locus	Fragment sizes (kb) Allele 1	Allele 2	No. of restriction sites
		Wood–plains bison		
*Eco*RI	GH	5.40		2
	IGF-1	9.80		4
		8.20		
		3.45		
	S	18.90		3
		3.20		
	GHRF	5.65		2
*Bam*HI	GH	13.90		2
	IGF-1	15.10		3
		13.00		
	GHRF	14.00		2
*Pst*I	GH	.64		2
	IGF-1	6.40		3
		5.90		
	GHRF	3.81		2
*Hin*dIII	GH	20.50		2
	S	20.54		3
		8.00		
	GHRF	8.75		2
*Pvu*II	GH	1.00		2
	S	21.00		2
*Xha*I	GH	13.70		2
	S	8.70		2
	GHRF	3.85		2
*Bgl*I	GHRF	7.00		2
*Dra*I	GHRF	6.95		2
*Eco*RV	GHRF	8.90 (E_1)	14.40 (E_2)	2
*Sph*I	GHRF	11.10 (S_1)	12.60 (S_2)	2
Total				50
		European bison		
*Eco*RV	GHRF	14.40		
*Sph*I	GHRF	11.10		

two loci; individual bison could be either homozygous or heterozygous for each of the *Eco*RV and *Sph*I fragments.

There was evidence for differentiation of fragment frequencies between wood and plains bison (Tables 3,4). The continuity of fragment frequencies between the bison populations indicated divergence at both *Eco*RV

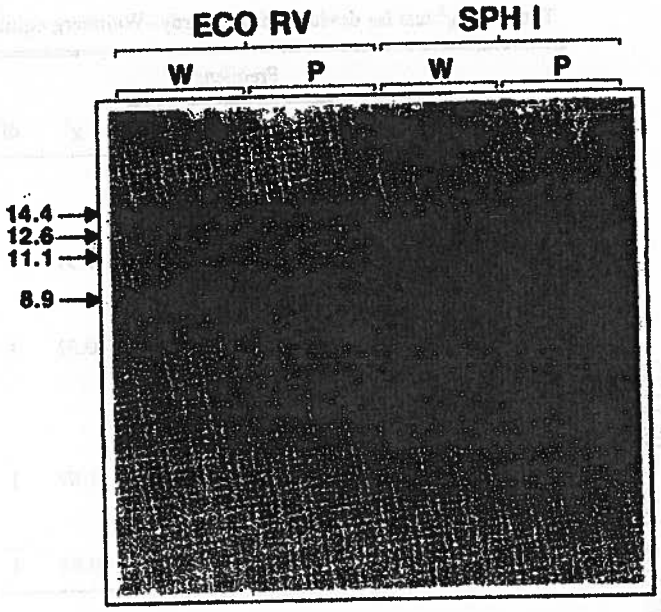

FIG. 1. Autoradiogram of the GHRF-related RFLPs within the wood (W) and plains (P) bison (for methodology, refer to Materials and methods). These polymorphic restriction fragments were revealed when bison DNA was digested with either *Eco*RV or *Sph*I. The size of the fragments are shown in kilobases on the left margin; *Sph*I fragments are 11.1 and 12.6 kb, the *Eco*RV fragments are 8.9 and 14.4 kb. Individual wood and plains bison could be either homozygous (single, dark-staining band) or heterozygous (two bands) for each of the *Eco*RV and *Sph*I restriction fragments.

TABLE 3. Frequency of alleles at polymorphic loci in wood and plains bison

		Population	
Enzyme	Locus	Wood ($N = 20$)	Plains ($N = 20$)
*Eco*RV	E_1	0.75	0.20
	E_2	0.25	0.80
*Sph*I	S_1	0.85	0.50
	S_2	0.15	0.50

($\chi^2 = 24.26$, 1 df, $p = 0.0$) and *Sph*I ($\chi^2 = 11.17$, 1 df, $p = 0.0$). Within wood bison, E_1 appeared at a higher frequency than E_2, whereas the reverse was true within the plains bison. At the dimorphic *Sph*I site, S_1 appeared at a higher frequency than S_2 within wood bison, whereas S_1 and S_2 appeared at equal frequency within the plains bison. The number of net nucleotide substitutions (DNA divergence) between the two populations was 0.0007.

The expected and observed genotypic frequencies at dimorphic sites within the experimental animals are presented in Table 4. The observed frequencies of the dimorphic sites did not differ from the Hardy–Weinberg expectations within either the wood or the plains bison.

A comparison of the North American bison GHRF-related restriction fragments with those identified in the four European bison samples is presented in Fig. 2. Both *Eco*RV and *Sph*I identify GHRF-related restriction fragments that were also identified within the North American species. The European bison *Eco*RV restriction fragment is 14.4 kb and the *Sph*I

TABLE 4. χ^2 test for deviation from Hardy–Weinberg equilibrium

Enzyme	Genotype	Frequency Observed	Frequency Expected	χ^2	df	p
		Wood bison				
*Eco*RV	E_1E_1	10	11.15			
	E_1E_2	10	7.69			
	E_2E_2	0	1.15	1.97	1	0.16
*Sph*I	S_1S_1	14	14.39			
	S_1S_1	6	5.23			
	S_2S_2	0	0.39	0.51	1	0.48
		Plains bison				
*Eco*RV	E_1E_1	0	0.72			
	E_1E_1	8	6.56			
	E_2E_2	12	12.72	1.07	1	0.30
*Sph*I	S_1S_1	4	4.87			
	S_1S_2	12	10.26			
	S_2S_2	4	4.87	0.61	1	0.44

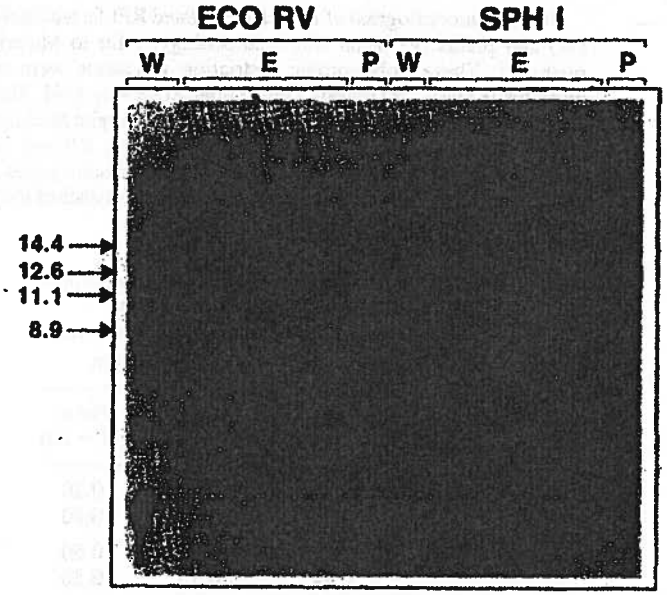

FIG. 2. Autoradiogram of the European (E) and North American (wood (W) and plains (P)) bison GHRF-related restriction fragments (for methodology, refer to Materials and methods). The size of the fragments are shown in kilobases in the left margin. When European bison DNA was digested with *Eco*RV, the GHRF locus yielded a restriction fragment of 14.4 kb, and digestion with *Sph*I yielded a fragment of 11.1 kb.

restriction fragment is 11.1 kb. The discovery of these fragments within the wisent implies that they are of primitive origin while the other restriction fragments (*Eco*RV 8.9 kb and *Sph*I 12.6 kb) discovered within the North American bison are the derived fragments (outgroup rule) (Wiley 1981, p. 139). It would have been beneficial to examine the wisent with the other 20 probe–enzyme combinations; however, analysis was restricted as a result of limited amounts of wisent DNA.

Discussion

Intrapopulation variability

[illegible] restriction site loci associated with growth-regulating genes, the genetic diversity within both the wood and plains bison populations at EINP appears to be low; polymorphisms were identified at only 2 of the 50 restriction site loci examined. This low level of intrapopulation genetic diversity is compatible with the history of the two populations; in the establishment of both the plains and the wood population, a very limited number of animals were available. The bison populations that exist today result from an original stock of 22 wood bison and 30 plains bison (Ogilivie 1979; Novakowski 1989). Since the populations are derived from a limited genetic foundation, the 20 animals sampled in this study probably provide a good indication of the variability present within bison.

While very few restriction site polymorphisms were identified within the wood and plains bison, the distribution of the identified polymorphic sites does reveal several structural features of the existing EINP populations. The Hardy–Weinberg equilibrium of the restriction site genotypes, in the wood and plains bison sampled (Table 4), implies that both bison populations are random mating; each male in the population appears to have an equal chance of mating with each female. Thus, although both populations have experienced bottlenecks in the past, our study suggests that the existing genetic information within each population is being evenly distributed.

Deviations from Hardy–Weinberg equilibrium may result if the allele frequencies in the small number of bulls mating differ from the allele frequencies in females (owing to sampling error). Therefore, the concept of random mating within each bison population may appear to be incompatible with the polygynous mating system typical of bison (Lott *et al.* 1987). The principal factor influencing this random distribution of genotypes is time. Both bison populations in EINP were established over 25 years ago, and this span encompasses the predominant lifetime of a bison. Within the polygynous mating system, selection for body size, weaponry, and behavior in males restricts monopolization of females to certain age classes (Jarman 1983). Although the age at which male bison may breed is a selective factor, over a lifetime most bulls will eventually mate, thus facilitating random mating within the bison populations.

Population structure of the EINP bison may also contribute to the randomized arrangement of genotypes. With the exception of the breeding season, bison bulls remain either solitary or roam in small temporary groups; cows form larger groups with the calves and young bulls. As the mating season approaches, the cows congregate into larger breeding clusters where they are joined by the mature bulls (Lott 1972, 1974, 1979). Therefore, depending upon the size of the breeding cluster relative to the total population size, a small number of dominant bulls may have access to a large proportion of the breeding females; the inability of many less dominant bulls to contribute to the gene pool could thereby result in genotypic frequencies which deviate from a Hardy–Weinberg equilibrium. However, Shackleton (1968) indicated that the group size of bison, within the wooded habitat of EINP, is smaller than it would be in open range. This smaller group size would increase the number of breeding clusters available. As a consequence, the less dominant bulls would have a greater probability to contribute to the gene pool, thereby increasing the opportunity to distribute genetic material uniformly throughout the population.

Random distribution of genotypes may also be attributed to the mobility of cows. Individual females are known to move rapidly and extensively through home ranges (Lott and Minta 1983). This thorough population mixing would increase each cow's chances of encountering different bulls in various years. Moreover, since the bulls are not associated with a specific herd

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Genetic relationships among North American bison populations

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Abstract: North American bison are presently divided into two subspecies: wood bison (*Bison bison athabascae*) and plains bison (*B. b. bison*). A survey was undertaken to determine the distribution of mitochondrial DNA haplotypes among subspecies and populations. Twelve haplotypes were identified with sequence data from the control region of mitochondrial DNA from 32 bison. Mitochondrial haplotypes for 269 bison from nine populations were then determined using the polymerase chain reaction and analyzed for restriction fragment length polymorphisms. Haplotype frequencies suggest genetic distances among bison populations from 0.0715 to 0.362. The extent of differentiation varies considerably. Based on the composition and phylogeny of haplotypes in the bison herds, plains bison form a paraphyletic group and wood bison form a polyphyletic group. Because neither subspecies of bison is derived from one lineage, neither is a well-defined taxon.

Résumé : En Amérique du Nord, le Bison compte actuellement deux sous-espèces, le Bison des bois (*Bison bison athabascae*) et le Bison des plaines (*B. b. bison*). Nous avons étudié la répartition des haplotypes d'ADN mitochondrial au sein des sous-espèces et populations. Douze haplotypes ont été identifiés au moyen des séquences de la région contrôle de l'ADN mitochondrial de 32 bisons. Les haplotypes de 269 bisons de neuf populations ont ensuite été déterminés par amplification au moyen de la réaction en chaîne par la polymérase et examinés pour y déceler le polymorphisme dans la longueur des fragments de restriction. D'après la fréquence des haplotypes, les distances génétiques entre les populations de bisons ont été estimées à 0,0715–0,362. L'importance de la différenciation varie considérablement. Selon la composition et la phylogénie des haplotypes dans les troupeaux de bisons, il semble que le Bison des plaines constitue un groupe paraphylétique et le Bison des bois, un groupe polyphylétique. Comme ni l'une ni l'autre de ces sous-espèces n'est issue d'une seule lignée, ces taxons restent très mal définis.
[Traduit par la Rédaction]

Introduction

Early conservation efforts contributed to the recovery of North American bison populations. Presently, the North American bison is divided into two subspecies: the wood bison (*Bison bison athabascae* Rhoads (1897)) and the plains bison (*B. b. bison*). Taxonomists have variously classified North American bison since they were first described by Hernán Cortés in 1519 (Allen 1876; McHugh 1972). The recent inquiry into the classification of bison was prompted by the issue of what to do with the diseased, free-roaming bison in Wood Buffalo National Park (WBNP), Northwest Territories and Alberta. The bison in WBNP are presently classified as hybrids between wood and plains bison (Geist 1991; McCormack 1992).

In 1993, an estimated 3000 "hybrid" bison resided in WBNP (Comin 1993), of which a large percentage were thought to have tuberculosis and brucellosis (Federal Environmental Assessment Review Panel (FEARP) 1990). The occurrence of brucellosis and tuberculosis in WBNP presented not only a danger to bison in the neighboring Mackenzie Bison Sanctuary, N.W.T., but also to cattle in ranches that have been established in the area. The hybrid bison from WBNP were also seen as a threat to the genetic purity of wood bison from the Mackenzie Bison Sanctuary, a herd whose range was beginning to encroach on the borders of WBNP. A suggestion by the Federal Environmental Assessment Review Panel (1990) to replace the extant population in WBNP with pure, healthy wood bison raised several issues regarding the genetic integrity of bison. Before eliminating the WBNP population, a genetic description of the wood bison was to be pursued, and a survey undertaken to determine the genetic variability of bison in the park.

To understand the present classification of bison, an overview of their history is necessary. Wood bison, described by Rhoads in 1897, ranged in the northern districts of Alberta, Saskatchewan, and Manitoba and throughout the Yukon and Northwest Territories. By 1914, the subspecies found in the area later known as Wood Buffalo National Park was reduced to 250 animals. Under the protection of the Northwest Mounted Police, the population in WBNP increased to more than 1500 animals by 1922 (FEARP 1990). During the time when the bison population in WBNP began to prosper, the bison population in Buffalo Park in Wainwright, Alberta, had risen beyond the park's capacity. From 1925 to 1929, about 6673 plains bison from Buffalo National Park (of Pablo-Allard herd origin) were relocated to the southern

Received April 5, 1995. Accepted October 23, 1995.

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Can. J. Zool. 74: 738–749 (1996). Printed in Canada / Imprimé au Canada

Table 1. An overview of the bison used to establish sanctuary, refuge, and park populations (Jenning and Hebbing 1983; Ogilvie 1979; Dary 1974; Bridges 1974; Rorabacher 1970; Haines 1970; Garretson 1938).

Population	Source	Origin	Year	No. of bison
Custer State Park	Philip	North Dakota	1914	30
	Sioux	na	1951	60
Elk Island National Park (plains)	Pablo-Allard	Alberta, Montana, Texas, Manitoba, Kansas, Nebraska	1909	50
Fort Niobrara Wildlife Refuge	Gilbert	Nebraska	1912	6
	Yellowstone	Yellowstone National Park	1913	2
	Yellowstone	Yellowstone National Park	1952	2
	Custer State	Custer State Park	1935	2
	Custer State	Custer State Park	1937	4
National Bison Range	Conrad	Pablo-Allard herd	1909	36
	Goodnight	South Dakota	1909	1
	Blue Mountain Forest	Manitoba, Texas	1910	13
	Conrad	Pablo-Allard herd	1910	3
	Yellowstone	Yellowstone National Park	1953	2
Wichita Mountains Wildlife Refuge	New York Zoological	Texas, South Dakota, Oklahoma, Wyoming	1907	15
	Fort Niobrara	Fort Niobrara Wildlife Refuge	1942	4
Yellowstone National Park	Yellowstone	Indigenous	1894	20
	Eton	Pablo-Allard herd	1902	na
	Goodnight	South Dakota	1902	3
Elk Island National Park (wood)	Wood Buffalo	Wood Buffalo National Park	1965	24
Mackenzie Bison Sanctuary	Wood Buffalo	Wood Buffalo National Park	1963	18
Wood Buffalo National Park	Wood Buffalo	Indigenous	1893	>300
	Buffalo National Park	Pablo-Allard herd, Goodnight herd, Saskatchewan, Manitoba	1925–1928	6673

region of WBNP. The presence of brucellosis and tuberculosis in WBNP was precipitated by the relocation of the plains bison. In 1957, a herd of presumed "pure" wood bison was found in WBNP (Novakowski 1979). In 1963 and 1965, 18 and 24 bison, respectively, were chosen from this herd to establish founding populations of wood bison at the Mackenzie Bison Sanctuary, N.W.T. (MBS) and Elk Island National Park, Alberta (Novakowski 1979).

Unlike the wood bison, which were reduced to one population by the early 1900s, fewer than 1000 plains bison were scattered across the central United States (Roe 1970). These plains bison were captured by ranchers and, together with their offspring, used to found the following public herds: Custer State Park, S.D., Elk Island National Park, Alberta (plains bison), Fort Niobrara Wildlife Refuge, Nebraska, National Bison Range, Montana, Wichita Mountains Wildlife Refuge, Oklahoma, and Yellowstone National Park, Wyoming. The transfer of ownership of private bison herds, acquisition of bison from several sources, and exchanges of breeding stock between parks have created a complex history for each park population (for an overview see Table 1).

Initially, morphological descriptions were used to separate the bison subspecies. However, Geist (1991), Berger and Peacock (1988), and McHugh (1972) warned that age- and sex-related differences, seasonal changes, general health, nutrition, population density, and individual variation could confound the use of characters such as pelage, body mass, and horn size. In addition, the classification of bison subspecies was based on one or few animals (Gavin 1988; Geist 1991). A shift from descriptive morphology to more quantitative morphometry did not clarify the problem of distinguishing the bison subspecies. Bayrock and Hillerud's (1965) and Shackelton et al.'s (1975) studies of cranial measurements showed a high degree of plasticity and variability. McDonald (1981) found that cranial, horn, and limb measurements of bison subspecies indicated a north–south cline effect. Alternatively, Van Zyll de Jong's (1986) and Van Zyll de Jong et al.'s (1995) use of external morphological characteristics and skeletal ratios suggested that the bison subspecies were genetically distinct.

Unable to resolve the status of bison subspecies by means of morphological characters, taxonomists turned to biochemical, molecular, and genetic characters. The first genetic studies employed to distinguish bison subspecies failed to find differences at the chromosomal and protein levels (Stormont et al. 1960; Wurster and Benirschke 1967; Johnston 1968; Ying and Peden 1977; Peden and Kraay 1979). Molecular studies of the growth hormone genes (Bork et al. 1991) and K-casein gene (Cronin and Cockett 1993) also failed to locate unique differences for bison subspecies.

Genetic studies to determine the relatedness of bison herds to each other are also limited (Peden and Kraay 1979; Bork et al. 1991; Geist 1991; Cronin and Cockett 1993; McCleneghan et al. 1990). In prior protein electrophoretic studies

(Wurster and Benirschke 1967; Ryman et al. 1980; Wooten and Smith 1984), values of heterozygosity were used to express the level of genetic variation in bison, but how different alleles were distributed among bison populations was not established.

Recently, the number of genetic variants, loci, and individuals that could be surveyed was expedited with the development of the polymerase chain reaction (PCR). Comparisons of molecular characters, i.e., nucleotide sequence or restriction site, have been employed to infer subspecies status (Cronin et al. 1994; O'Brien and Menotti-Raymond 1993), establish biogeographical boundaries (Avise et al. 1987; Gilbert et al. 1990; Thomas et al. 1990), and identify historical distributions (Kessler and Avise 1985; Diamond 1990). Phenetic and cladistic analyses of molecular characters have also been used to establish evolutionary relationships between individuals, populations, and subspecies (Nei 1987; Avise 1989; Bernatchez and Dodson 1991; Cronin 1992).

Sequence data from PCR products have been used to estimate genetic distances between populations; however, as sequencing is time consuming and expensive, only a few studies with this type of population data exist (Meyer et al. 1990; Thomas et al. 1990; Vigilant et al. 1991). An alternative method of surveying DNA variation in a population consists of first sequencing the DNA from a small number of individuals and then surveying those variants that are recognized by restriction enzymes in a large number of individuals (Kreitman and Aguade 1986; Cann et al. 1987). This method, however, has two disadvantages. First, only a small fraction of the observed variants in the population can be assayed using known restriction enzymes. Second, the variant that causes the loss of the restriction site cannot be identified because any mutation in the restriction recognition sequence will result in a deletion of the restriction site.

Allele-specific PCR (Wu et al. 1989; Sommer et al. 1992) was developed so that all nucleotide variants could be assayed, not just those causing restriction site changes. In this method, the primer sequence is modified at the 3′ end to match the nucleotide changes in each allele. Ideally, a PCR product will be produced if, and only if, a particular variant is present; however, alterations in the primer sequence may not prevent similar alleles from amplifying. Therefore, Lien et al. (1992) and Todd and Iland (1991) designed primers that always produce PCR products but generate restriction sites only when specific nucleotides are present at the variable sites. This method overcomes the disadvantages of using restriction enzymes alone or allele-specific PCR to survey DNA variation in natural populations, i.e., all variants can be precisely and accurately surveyed. To date, no natural populations have been surveyed using the primer-generating restriction fragment length polymorphism (PG-RFLP) technique.

While many approaches have been taken to find characters that can separate bison subspecies, not one diagnostic character has been found. O'Brien and Mayr (1991) suggested that because subspecies are often reproductively compatible, and may have overlapping ranges, their status could be determined from their phylogenetic relationships. Well-defined subspecies are preferably derived from one or two recent common ancestors and are described as mono- or para-phyletic, while poorly defined subspecies would contain individuals originating from several lineages and are described as polyphyletic (Simpson 1990, p. 124).

A survey of PG-RFLPs and standard RFLPs from the control region of mtDNA from nine bison populations was used to establish the genetic distance between bison populations and to infer the phylogenetic relationship of wood and plains bison. Variable sites suitable for PG-RFLP and standard RFLP analysis were chosen from sequence data from 32 bison.

Materials and methods

Collection

Blood was collected from the following immobilized bison by Parks Canada personnel: 40 plains bison and 45 wood bison at Elk Island National Park (EINP), 22 bison from MBS, and 58 bison from four areas of WBNP (Alberta and N.W.T.), Sweetgrass (21), Pine Lake (21), Garden Creek (8), and Little Buffalo (8). The United States Fish and Wildlife Service also provided 12 samples from Yellowstone National Park (YNP), 22 from National Bison Range (NBR), 20 from Fort Niobrara Wildlife Refuge (FNWR), 20 from Wichita Mountains Wildlife Reserve (WMWR), and 30 from Custer State Park, S.D. (CSP). All samples were collected in Vacutainers™ containing EDTA or heparin, shipped on ice, and stored at 4°C until processing. DNA was isolated from whole blood by standard organic extractions as described in Bork et al. (1991).

Amplification (PCR)

The control region of mtDNA was enzymatically amplified in 100 μL of a solution containing 0.06 mM each of dATP, dTTP, dCTP, and dGTP, 1 × *Taq* magnesium-free polymerase buffer (Promega, Madison, Wis.), 2.0 μM magnesium chloride, 20 pM each of primers CST 39 and CST 2, genomic DNA (10–1000 ng), and 1 unit of *Taq* polymerase, and brought up to volume with sterile double-distilled water. Amplification was performed on a PHC-2 Techne Thermocyler with the following conditions: 94°C for 5 min, 54°C for 30 s, and 72°C for 2 min, followed by 30 cycles of 94°C for 15 s, 54°C for 30 s, and 72°C for 2 min, ending with 94°C for 15 s, 54°C for 30 s, and 72°C for 10 min. Sequencing reactions of the control region were performed as described in the T7 Sequencing kit™ (Pharmacia Laboratories, Piscataway, N.J.) whereby 5 μL of each reaction was loaded on a 8% polyacrylamide 8 M urea gel.

We used primer sequences flanking the control region based on those described by Kocher et al. (1989) and biased for domestic cow (Anderson et al. 1982): CST 2 (5′-TAATATACTGGTCTTGTAA-ACC-3′) and CST 39 (5′-GGGTCGGAAGGCTGGGACCAAACC-3′). The sequence for the bison control region (Fig. 1) (NCBI Genbank U12933–U12960) was used to design internal and allele-specific primers (Table 2). The internal and allele-specific primers were used to amplify regions containing informative sites using the conditions described above.

Primer-generated restriction fragment length polymorphisms

In total, 12 sites found to be variable from sequence data could be analyzed by restriction enzymes: 4 sites, 221, 289, 429, and 607, were recognized by means of standard RFLP and 8 sites by means of PG-RFLPs (Table 3). Primers used to amplify sections of the control region, the enzymes used to assay each variable site (shown in boldface type), and the restriction fragment patterns corresponding to each allelic variant are also given in Table 3. All restriction digests used 100–200 ng of amplified mtDNA and 2 units of enzyme. To compensate for salt residues present in the amplified products, buffer concentrations recommended by the manufacturer

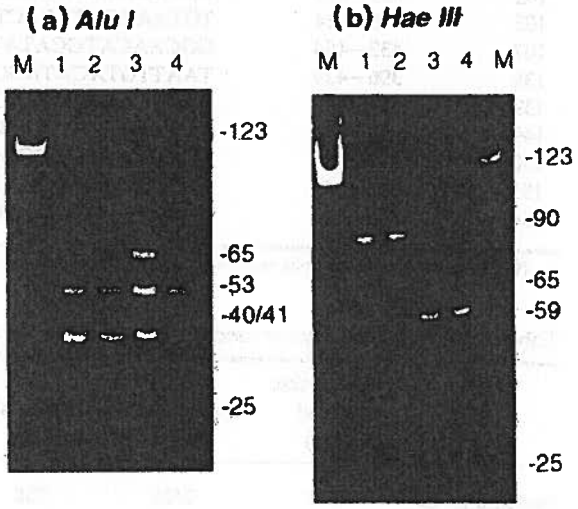
Fig. 1. Consensus sequence from the control region of mtDNA of 32 bison; variants are given above the line and deletions are indicated by (-).

```
                                                                    50
TAATATACTG GTCTTGTAAA CCAGAAAAGG AGAGCAACTA ACCTCCCTAA
                                                                   100
GACTCAAGGA AGAAACTACA GTCTCACCGT CAACCCCCAA AGCTGAAGTT
                                                                   150
CTATTTAAAC TATTCCCTGA ACGCTATTAA TATAGTTCCA TAAATGCAAA
                                                  G                200
GAGCCTCACC AGTATTAAAT TTACTAAAAA TTCCAATAAC TCAACACAAA
          C                                                        250
CTTTGTACTC TAACCAAATA TTGCAAACAC CACTAGCTAA CGTCACTCAC
                                                  T                300
CCCCAAAATG CATTACCCAA ACGGGGGAAT ATACATAACA TTAATGTAAT
                                                                   350
AAAAACATAT TATGTATATA GTACATTAAA TTATATGCCC CATGCATATA
                TT        A T                                    400
AGCAAGTACT TATCCTCTAT TGACAGTACA TAGTACATAA AGTTATTAAT
                                  C T                        C 450
TGTACATAGC ACATTATGTC AAATCTACCC TTGGCAACAT GCATATCCCT
     C                    C                                     500
TCCATTAGAT CACGAGCTTA ATTACCATGC CGCGTGAAACC AGCAACCCG
                                                                   550
CTAGGCAGAG GATCCCTCTT CTCGCTCCGG GCCCATGAAC CGTGGGGGTC
                T                                                  600
GCTATTTAAT GAACTTTATC AGACATCTGG TTCTTTCTTC AGGGCCATCT
       A                                                           650
CACCTAGAAT CGCCCATTCT TTCCTCTTAA ATAAGACATC TCGATGGACT
     A                                                             700
AATGGCTAAT CAGCCCATGC TCACACATAA CTGTGCTGTC ATACATTTGG
                                                                   750
TATTTTTTTA TTTTGGGGGA TGCTTGGACT CAGCTATGGC CGTCAAAGGC
                                                                   800
CCTGACCCGG AGCATCTATT GTAGCTGGAC TTAACTGCAC CTTGAGCACC
            G                                           -  T
AGCATAATGG TAAGCATGGA CATATAGTCA ATGGTTACAG GACATAAACT
            -                                                      900
GTATTATATA TCCCCCCCTC CATAAAAATT CCCCCTTAAA TATTTACCAC
                                                                   950
TGCTTTTAAC AGATTTTTCC CTAGTTACCT ATTTAAATTT TCCACACTTT
                                                                  1000
CAATACTCAA ATTAGCACTC CATATAAAGTC AATATATAA ACGCAGGCCC
                                                                  1050
CCCCCCCCCG TTGATGTAGC TTAACCCAAA GCAAGGCACT GAAAATGCCT
                                                                  1100
AGATGAGTCT CCCAACTCCA TAAACACATA GGTTTGGTCC CAGCCTTCCG
```

were decreased in magnitude from high to medium, medium to low, and low to zero. The digested DNAs were separated by electrophoresis in a BioRad vertical gel apparatus (BioRad, Richmond, Calif.) on 4 or 6% polyacrylamide 1 × TBE gels (Sambrook et al. 1989), stained, and photographed.

The development of the primer-generated RFLP technique (Wu et al. 1989; Lien et al. 1992) allowed sites 364, 365, 427, 446, 455, 564, 655, and 813 to be assayed. Primers used to generate restriction sites are summarized in Table 4, where regions of the primers partially containing the restriction sites are highlighted. Primers

Fig. 2. PG-RFLPs for variants at site 813 in bison. A 159-bp PCR product is produced when the primer 5'-CTGTGCTGTCATACATTTGG-3' is used with either primer CST 102 or primer CST 103. (*a*) Digestion of the PCR product using CST 102 with *Alu*I produces fragments 53, 41, 40, and 25 bp in length *if, and only if,* there is a T at site 813 (1 and 2) and 65, 53, and 41 bp if there is a C at site 813 (3 and 4). (*b*) Digestion of the PCR product using CST 103 with *Hae*III produces fragments of 90, 50, and 10 bp *if, and only if,* there is a T at site 813 (1 and 2) and 65, 59, 25, and 10 bp if there is a C at site 813 (3 and 4). The PCR products were run on a 8% polyacrylamide 1 × TBE gel and stained with ethidium bromide.



used to amplify regions containing sites 365, 655, and 813 were designed to anneal to the complementary DNA strand. All samples were amplified in 100-μL volumes and restricted as previously described. Products less than 250 base pairs (bp) long were separated on a 8% 1 × TBE acrylamide vertical gel, whereas larger products were separated on a 6% TBE acrylamide gel.

In the PG-RFLP method, primers are designed to anneal to the template directly before the variable nucleotide. Usually only one nucleotide of the primer sequence was mismatched to the template to create a restriction recognition site. The initial three bases of a four-base recognition site (e.g., AGC of the *Alu*I site AGCT) were included in the primer sequence, while the fourth base was determined by the DNA template (position 813). If the first nucleotide of the template was a T, the recognition sequence for *Alu*I would be completed and the amplified DNA could be cleaved. Fragments resulting from the digestion of DNA amplified from the haplotype with the *Alu*I site at the 813-bp position would be 53, 41, 40, and 25 bp in length, as indicated in Fig. 2. Any other nucleotide found at this position woud result in the absences of the *Alu*I site. PCR amplification and subsequent digestion of DNA from haplotypes without this *Alu*I site would result in fragment lengths of 65, 53, and 41 bp as shown in Fig. 2. Although amplifed samples that were cleaved had a T at site 813, they were designated the complementary A allele to correspond to the sequence data.

To confirm the identity of the G allele at site 813, primers were constructed to create a *Hae*III site in the presence of the G allele. *Hae*III recognizes the sequence GGCC, and once again the first three nucleotides of this sequence were incorporated into the primer. When the A allele was present, the template would continue

Table 2. PCR primers used to assay variable sites in the control region of *Bison bison*.

Primer	Location (bp)	Sequence primer (5′–3′)
2	1–22	TAATATACTGGTCTTGTAAACC
16	218–240	ATATTGCAAACACCACTAGCTAAC
18	814–792	CTTATCATTATGCTGGTGCTCAAG
24	681–700	CTGTGCTGTCATACATTTGG
25	538–515	TCATGGGCCGGAGCGAGAAGAGG
29	483–504	CGTGAAACCAGCAACCCGCTAGGC
39	1104–1081	GGGTCGGAAGGCTGGGACCAAACC
84	595–572	GCCCTGAAGAAAGAACCAGATGTC
102	839–814	TGTAACCATTGACTATATGTGCAAGC
103	839–814	TGTAACCATTGACTATATGTGCAGGC
107	433–454	GGCAACATGCATATCCCTTTCT
138	396–426	TAATTGTACATAGCACATTATGTCAAATGT
139	415–445	ATGTCAAATCTACCCTTGGCAACATGCGTA
149	686–656	AGCACAGTTATGTGAGCATGGGCTGATTGG
150	533–563	CATGAACCGTGGGGGTCGCTATTTAATGAT
155	333–363	TATGCCCCATGCATATAAGCAAGTAAATAT
156	396–366	TAACTTTATGTACTATGTACTGTCACTCGA

Note: The underlined regions represent primers that have a template-dependent restriction site.

Table 3. Primers and enzymes used to assay variable sites in the bison control region.

	No. of sites tested (bp)	Primer pair (A/B)	Product size (bp)	Enzyme	Sequence recognized by enzyme	Variant tested	Size of fragment (bp)
Standard RFLP	221	2/25	538	*Ssp*I	AAT′ ATT	C	538
						T	538 or 289, 249
Standard RFLP	289	2/25	538	*Ssp*I	AAT′ ATT	C	538
						T	289, 249 or 249, 219, 70
PG-RFLP	364	155/25	205	*Ssp*I	AAT′ ATT	C	205
						T	175, 30
PG-RFLP	365	16/156	178	*Xho*I	C′ TCGAG	C	148, 30
						T	178
PG-RFLP	427	138/25	142	*Rsa*I	GT′ AC	A	112, 23, 7
						T	135, 7
Standard RFLP	429	16/39	886	*Sty*I	C ′CTAGG	C	675, 211
						T	886
PG-RFLP	446	139/25	123	*Rsa*I	GT′ AC	C	94, 29
						T	123
PG-RFLP	455	107/84	162	*Xba*I	T′ CTAGA	C	141, 21
						T	162
PG-RFLP	564	150/18	281	*Sau*3A	′ GATC	C	252, 29
						T	281
Standard RFLP	607	16/39	886	*Hin*fI	G′ ANTC	G	497, 389
						A	886
PG-RFLP	655	29/149	203	*Hae*III	GG′ CC	G	63, 61, 48, 31
						A	92, 63, 48
PG-RFLP	813	24/102	158	*Alu*I	AG′ CT	G	65, 53, 41
						A	53, 41, 40, 25
PG-RFLP	813	24/103	158	*Hae*III	GG′ CC	G	65, 59, 25
						A	90, 59

Note: Variable sites are shown in boldface type.

Table 4. Designation of *Bison bison* haplotypes based on variants found from sequencing 32 bison and surveyed using PG-RFLP and standard RFLP analysis.

	Nucleotide variants at site:											
Haplotype	221	289	364	365	427	429	446	455	564	607	655	813
COW	T	C	C	C	A	T	T	T	T	A	G	A
1	T	C	C	C	A	T	T	T	C	A	G	A
2	T	C	C	C	A	T	T	T	C	G	G	G
3	T	C	C	C	A	C	T	T	C	G	G	G
4	T	C	C	C	A	C	T	T	C	G	G	A
5	T	T	C	C	A	C	T	T	C	G	G	A
6	T	C	T	C	A	C	T	T	C	G	G	A
7	T	C	C	C	A	C	T	T	T	G	G	A
8	T	C	C	C	A	C	T	T	C	A	G	A
9	T	C	C	C	A	C	T	T	C	A	A	A
10	C	C	C	C	A	C	C	T	C	A	G	A
11	C	C	C	T	A	C	C	T	C	A	G	A

to read GGCT, while the G allele would continue to read GGCC. The fragments resulting from the *Hae*III digests are given in Fig. 2.

Phylogenetic analysis

The nucleotide substitutions were identified from sequence data and assayed in each of the 269 bison with PG-RFLPs or standard restriction digests (RFLPs). Each haplotype represented a set of restriction patterns that described the 12 sites assayed (Table 4). The relationships of the mtDNA haplotypes were assessed by the branch and bound option of the computer package PAUP 3.0 (phylogenetic analysis using parsimony) (Swofford 1991). Trees were rooted using *Bos taurus* and examined for para- and polyphyly.

Population analysis

Haplotype frequencies were calculated for all bison populations. Populations were further analyzed for intrapopulation (eq. 1) and interpopulation (eqs. 2 and 3) distances by adopting the equation for minimum genetic distance from Nei and Li (1979) and Nei (1987):

$$[1]\quad D_{XX} = \sum_{i=1}^{n}\sum_{j=1}^{n}\frac{N_i N_j d_{ij}}{N_T^2}$$

$$[2]\quad D_T = \sum_{i=1}^{n}\sum_{j=1}^{n} x_i y_j d_{ij}$$

$$[3]\quad D_{XY} = D_T - \left(\frac{D_{XX} + D_{YY}}{2}\right)$$

where N_i and N_j are the number of individuals with each haplotype i and j, respectively; d_{ij} is the number of nucleotide differences between the haplotypes; N_T is the total number of individuals in the population; D_T is the total distance between all haplotypes in populations X and Y; x_i and y_j are the frequencies of each haplotype; $(D_{XX} + D_{YY})/2$ is the average distance within populations X and Y; and D_{XY} is the minimum genetic distance between populations X and Y. These distances were used to create an unrooted population distance tree using the computer application Fitch in PHYLIP 3.5c (phylogenetic inference package; Felsenstein 1993). Fitch groups least distant populations through a sequential clustering algorithm. Statistical differences between populations were calculated using analysis of molecular variants (AMOVA; Excoffier et al. 1992).

Results

The control region of mtDNA in bison consisted of 1105 nucleotides with 21 polymorphic sites (Genbank U12933–U12960). These included 4 variable sites (183, 364, 427, and 655) unique to individuals and 17 sites (221, 273, 278, 289, 365, 372, 374, 429, 446, 455, 472, 564, 607, 813, 846, 849, and 861) with variants shared among individuals. With the exception of site 374, only 12 of the 21 variable sites were suitable for standard or PG-RFLP analysis. Sites 221, 289, 429, and 607 were surveyed using standard RFLP analysis and sites 364, 365, 427, 446, 455, 564, 655, and 813 by the PG-RFLP technique. Sites 364 and 655 were found to be shared among individuals and contributed to haplotype grouping. The unique change at site 427 identified in the sequence data was invariable in all other bison. Only sites 221, 429, 607, and 813 were considered to be phylogenetically informative.

Combinations of the variants from the 12 sites surveyed were compiled and 11 haplotypes were identified (Table 4). Because the control region is only a fraction of the total mtDNA, the 11 haplotypes identified from this region do not represent the total number of bison haplotypes in existence.

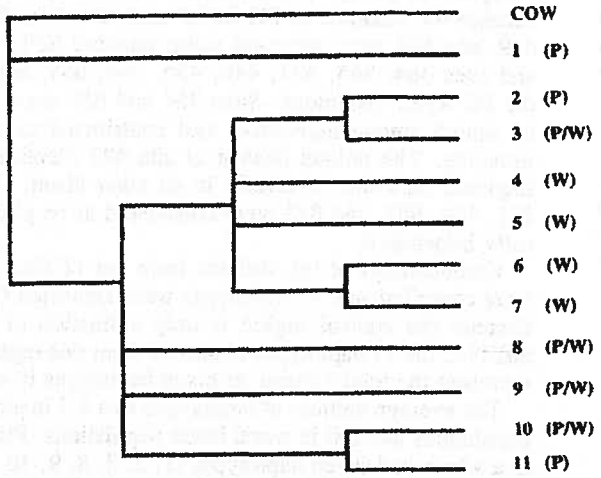
The average number of haplotypes was 3.3 in plains bison populations and 6.0 in wood bison populations. Plains bison as a whole had seven haplotypes (1, 2, 3, 8, 9, 10, and 11). Haplotypes 1 and 2 were unique to CSP. Haplotype 1 was also seen in *Bos taurus*, and from sequence data, haplotype 1 was shown to vary at 80 nucleotides from the bison consensus sequence and at one nucleotide from the *B. taurus* sequence (Polziehn et al. 1995). Wood bison herds had eight haplotypes (3, 4, 5, 6, 7, 8, 9, and 10). Haplotypes 3, 8, 9, and 10 were shared by the two subspecies, while haplotypes 4, 5, 6, and 7 were unique to wood bison from EINP, MBS, and WBNP.

Only one parsimonious tree (Fig. 3) was constructed for the 11 bison haplotypes using PAUP 3.0. The gene tree was based on four informative and seven unique unweighted characters, and rooted using *B. taurus*. The consistency index (CI) and branch length for the tree were 0.846 and 13, respectively. Reversals of nucleotides occurring along the

Table 5. Mitochondrial DNA haplotype frequencies (%) observed in bison populations from North America.

Population and subpopulation	mtDNA haplotype 1	2	3	4	5	6	7	8	9	10	11	*n*
Yellowstone National Park	—	—	25.0	—	—	—	—	—	—	—	75.0	12
National Bison Range	—	—	31.8	—	—	—	—	13.6	13.6	—	40.9	22
Custer State Park	6.7	30.0	26.7	—	—	—	—	—	3.3	—	33.3	30
Fort Niobrara Wildlife Refuge	—	—	—	—	—	—	—	—	—	100.0	—	20
Wichita Mountains Wildlife Refuge	—	—	5.0	—	—	—	—	55.0	—	40.0	—	20
Elk Island National Park (plains)	—	—	52.5	—	—	—	—	2.5	15.0	12.5	17.5	40
Elk Island National Park (wood)	—	—	22.2	8.9	26.7	20.0	22.2	—	—	—	—	45
Mackenzie Bison Sanctuary	—	—	4.5	—	36.4	—	18.2	9.1	—	31.8	—	22
Wood Buffalo National Park	—	—	17.2	6.9	22.4	8.6	17.2	3.4	10.3	13.8	—	58
Pine Lake	—	—	19.0	4.8	33.3	9.5	14.2	—	9.5	9.5	—	21
Sweetgrass	—	—	14.3	14.3	9.5	14.3	9.5	9.5	14.2	14.2	—	21
Little Buffalo	—	—	12.5	—	25.0	—	37.5	—	12.5	12.5	—	8
Garden Creek	—	—	25.0	—	25.0	—	25.0	—	—	25.0	—	8

Fig. 3. A phylogeny for bison resulting from parsimonious analysis of mtDNA control region haplotypes. Haplotypes 1–11 were identified by primer-generated RFLPs or restriction analysis, and correspond to haplotypes described in Table 2. Haplotypes found in individuals recognized as wood or plains bison were designated as wood (W) and plains (P). The tree has a branch length of 13 and CI of 0.846. Domestic cow (COW) was identified as the out-group.

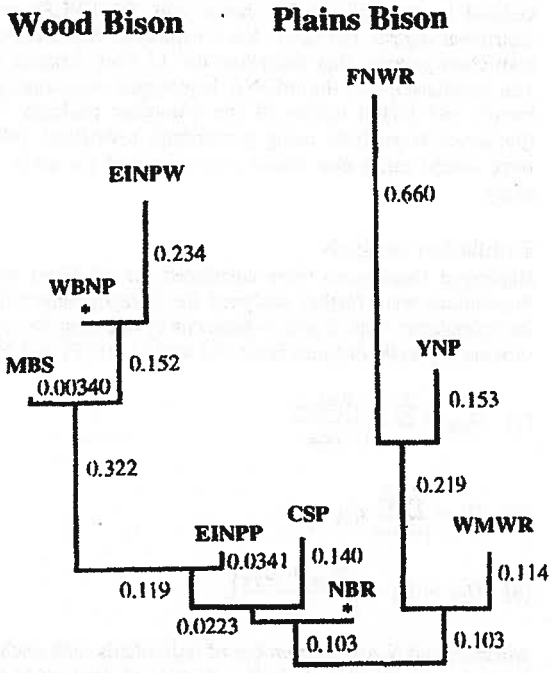


branches for cow and haplotype 7 at site 564, and along branches separating groups 1 and 2 at site 429, lowered the CI and increased the branch length. Because all other branch lengths had a CI of 1.00, bootstrapping was not performed to determine confidence levels.

Gene trees are used to infer phylogenetic relationships where each taxonomic group is assumed to have independently evolving loci and, over time, a unique set of alleles. From the phylogeny, plains bison were found to be a paraphyletic group and wood bison to be a polyphyletic group. Not all lineages derived from the most recent common ancestor were included in their respective groups. If considered subspecies, both plains and wood bison would have had to evolve two and three haplotypes parallel to each other, respectively.

[illegible] ncies varied within and between herds

Fig. 4. Genetic distances between North American bison populations based on Fitch analysis of haplotypes identified by restriction analysis of 12 characters. An asterisk denotes a branch distance of 0.000. For an explanation of abbreviations see Table 6.



(Table 5). Bison in FNWR were fixed for one haplotype, while bison at WBNP had eight haplotypes. Bison populations sharing founding stock also did not express the same distribution or number of haplotypes. The differences in the distributions of haplotypes in populations founded from similar populations are likely due to random drift and unequal representation of haplotypes in the founding stock. For example, wood bison from EINP did not share haplotypes 8, 9, or 10 with those from WBNP, and likewise those from MBS did not share haplotypes 4, 6, and 9 with those from WBNP, even though they were captured from the same area.

While WBNP had the most haplotypes, the distribution of

Table 6. Minimum genetic distance as determined by haplotype frequency from North American bison populations using pairwise comparisons.

	YNP	NBR	CSP	WMWR	FNWR	MBS	WBNP	EINPP
NBR	0.376							
CSP	0.937	0.143						
WMWR	0.887	0.512	0.870					
FNWR	0.812	1.087	1.925	0.725				
MBS	1.282	0.528	0.789	0.495	1.470			
WBNP	1.720	0.701	0.564	0.784	2.218	0.146		
EINPP	0.916	0.165	0.296	0.500	1.585	0.478	0.396	
EINPW	2.506	1.108	1.194	1.569	3.388	0.501	0.184	0.890

Note: CSP, Custer State Park; EINPP and EINPW, Elk Island National Park plains and wood bison, respectively; FNWR, Fort Niobrara Wildlife Refuge; MBS, Mackenzie Bison Sanctuary; NBR, National Bison Range; WBNP, Wood Buffalo National Park; WMWR, Wichita Mountains Wildlife Refuge; YNP, Yellowstone National Park.

Table 7. Probabilities that random distances are greater than observed differences between bison populations using AMOVA.

	NBR	CSP	WMWR	FNWR	MBS	WBNP	EINPP	EINPW
YNP	0.0839	0.0110	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
NBR		0.0719	0.0410	0.0000	0.0050	0.0000	0.1518	0.0000
CSP			0.0040	0.0000	0.0000	0.0000	0.1039	0.0000
WMWR				0.0000	0.0060	0.0000	0.0040	0.0000
FNWR					0.0000	0.0000	0.0000	0.0000
MBS						0.1279	0.0040	0.0000
WBNP							0.0000	0.0020
EINPP								0.0000

Note: Values below 0.05 are statistically different at the 5% level. For an explanation of abbreviations see Table 6.

the haplotypes was not significantly different among subpopulations in WBNP (contingency $\chi^2 = 14.357$, corrected for small sample size; $p > 0.80$, 21 df; Spiess 1977). The Pine Lake and Sweetgrass subpopulations contained haplotypes 3, 4, 5, 6, 7, 8, 9, and 10, of which genotypes 4, 6, 8, and 9 were missing from the subpopulations at Garden Creek and Little Buffalo. The absence of haplotypes likely corresponds to the smaller sample size taken from Garden Creek and Little Buffalo. WBNP bison did not share haplotype 8 or 10 with wood bison at EINP, haplotype 4 or 6 with wood bison at MBS, or haplotype 9 with either wood bison population.

From pairwise comparison, genetic distances within populations ranged from 0.000 for FNWR to 2.684 for CSP. With the exclusion of FNWR, the average pairwise differences within populations were not significantly different. The average pairwise differences among populations of wood, plains, and WBNP bison populations were 2.129, 2.202, and 2.495, respectively.

Minimum genetic distances between bison populations, corrected for intrapopulation variation, are given in Table 6. An unrooted population distance tree was derived from Fitch (Fig. 4) with a standard deviation of 28.33 and a least sum of squares of 5.620. The most similar populations were NBR and CSP ($d = 0.140$) and WBNP and MBS ($d = 0.156$), while the least similar populations were FNWR and EINP wood bison ($d = 1.933$). EINP plains bison ($d = 0.153$) and WMWR bison ($d = 0.320$) were the next closest populations through grouping closest to FNWR ($d =$ 0.812), the YNP population had a distance of 0.4879 from WMWR.

Most populations were found to be significantly different ($p < 0.005$) from each other using AMOVA (Table 7). At $p > 0.05$, the NBR population was not different from the EINP plains bison, YNP, or CSP populations, the MBS population was not different from that at WBNP, and EINP plains bison were not different from the CSP population.

Discussion

Sequence data were useful for identifying nucleotide changes within the control region of mtDNA in bison, but unless many individuals were sequenced, the extent to which these changes were shared among bison populations would be unknown. A survey of nine bison populations for these variants with standard RFLP and PG-RFLP analyses identified the distribution of mtDNA haplotypes Haplotypes ranged from common (haplotype 3) to exclusive to individual parks (haplotypes 1 and 2).

Typical restriction digests gave unambiguous banding patterns where it was assumed that the cleaved DNA would represent one of the two variants identified from the consensus sequence. In reality, the possibility exists that an enzyme did not restrict the DNA because a change in any one of the nucleotides found at the recognition sequence could cause the site to be unrecognized. Because there were few changes in the control region and no more than one variant was found at any such site, the likelihood of incorrectly classifying a haplotype using this technique was low.

The amount of variation in the maternally inherited mtDNA varies among species. Shields et al. (1993) found that 6.1% (37 haplotypes over 360 nucleotides) varied in the control region of humans, Irwin et al. (1991) found that 1.5% (17 of 369 nucleotides) varied in the cytochrome *b* region of dolphins (*Stenella longirostris*), and Dorozynski (1994) stated that there was a 7% difference in the cytochrome *b* sequence of European bears, *Ursus arctos*. The presence of only 11 haplotypes over 1100 nucleotides in bison likely results from population bottlenecks, where the number of North American bison was severely reduced during the Ice Age and again when the continent was colonized by European settlers.

Because bison historically occupied most of North America and herds were not confined by geographical barriers, gene flow between populations likely was uninterrupted. The presence of universal haplotype 3, and to a lesser extent haplotypes 8, 9, and 10, suggests that prior to the extirpation of bison, gene flow had occurred between populations. The presence of the same genotypes within two populations implies that movement had occurred or is occurring between the two groups (Kocher et al. 1989; Thomas et al. 1990). The bison population in WBNP consists of widely dispersed herds, but individuals are not restricted to staying within one subpopulation. The similar distribution of haplotypes in four subpopulations in WBNP supports unrestricted gene flow as a result of random mating.

Haplotype frequencies varied between parks to the extent that most parks were significantly different from each other. Selection pressures may cause haplotype frequencies to vary in each park, but more likely the differences resulted from sampling error and founder effect. Sampling error occurs when samples are taken from individuals in only one subpopulation or area of a range. Such samples may not represent the entire population if extensive gene flow is not occurring between herds, i.e., daughters are not dispersing to different regions of the park. The number of founders will also affect the rate at which alleles are randomly lost or fixed. Because the largest number of founders (>250) to create a population was at WBNP (FEARP 1990), one may expect WBNP to have the greatest diversity in haplotypes and to be the least affected by genetic drift.

The presence of unique haplotypes 4, 5, 6, and 7 in bison herds originating from the north has at least two possible explanations. First, the origin of the northern haplotypes may be linked to the distribution of earlier forms of bison. Bison appeared in southern and eastern Asia about 2.5 million years BP (McHugh 1972), and later moved northward across Siberia into North America (Guthrie 1966). Two species of bison, *Bison priscus* and *Bison antiquus*, became established on the Bering land bridge, where the former is suggested to have given rise to *Bison occidentalis* (Pielou 1991). Present-day North American bison are a hybrid of *B. antiquus*, which moved southward through the ice-free Rocky Mountain corridor or along the west coast refugia, and *B. occidentalis*, which remained in the north (Guthrie 1970). Because modern bison probably originated only 5000 years ago (Wilson 1969; Van Zyll de Jong 1986), the north–south split between bison subspecies could be explained if wood bison consisted largely of *B. occidentalis* and plains bison (*B. b. bison*) consisted of mostly *B. antiquus*.

The second possibility is that the northern haplotypes were not limited to northern bison, but were present in all bison populations, albeit in lower frequencies. The elimination of bison herds between the northern and southern ranges of bison in the late 1800s destroyed the source of bison that could demonstrate the existence of a cline. Fossil records, however, indicate that plains bison types were found in the vicinity of the traditional wood bison range (Van Zyll de Jong 1986; C.R. Harington, personal communication), and wood bison types were located in the northern region of the plains bison range (C.R. Harington, personal communication). The overlapping ranges of bison present the possibility of a cline between northern and southern bison. To rule out the possibility that the unique haplotypes were present in plains bison herds before they were destroyed, the survey of bison has been recently extended to include mtDNA in skeletal remains from bison prior to the 1900s (C. Chambers, personal communication).

In the phylogenetic trees created from mtDNA haplotypes, wood and plains bison were not separated into distinct phylogenetic groups. Although four unique haplotypes exist in bison at WBNP, EINP, and MBS, five haplotypes were shared between the subspecies. If the shared haplotypes were not already present in WBNP, their presence suggests that the introduced plains bison became highly dispersed among the WBNP population or that the wood bison in the Nyarling River region were not as isolated as was first believed (Soper 1941). The presence of tuberculosis among EINP wood bison founders also implies the presence of infected plains bison in this region. Differences in haplotype frequencies for EINP and MBS populations likely arose from selecting founding bison over two separate sampling periods.

The presence of unique plains bison haplotypes in WBNP would imply that plains bison haplotypes were indigenous to the park; however, their absence does not suggest the opposite. Unfortunately, the introduction of plains bison to WBNP has destroyed the ability to determine if the plains bison haplotypes were already present in the northern populations or were introduced. The presence of plains haplotypes in the wood bison populations as a result of hybridization or lineage sorting cannot be determined.

Detecting hybridization is most effective if there are significant differences between the different lineages and most members of a taxa have lineages derived from one clade. Bison haplotypes differ by no more than a few nucleotides. Lineage sorting, which can result in phylogenies where taxa appear to be para- or poly-phyletic, will require long periods of time before fixation of genotypes will produce monophyletic groups. The lack of monophyly, complicated by human intervention, suggests that the bison subspecies have only recently been separated from each other. Because gene trees may not reflect species trees if populations are separated for short periods of time, including information from nuclear loci is suggested (Pamilo and Nei 1988). The presence of unique northern nuclear genotypes would lend support to the recent separation of the wood and plains bison subspecies.

As the populations presently exist, bison in WBNP, MBS, and EINP have a common gene pool. The proposal to replace WBNP with "pure wood" bison from EINP or MBS was based on the belief that populations at MBS and EINP con-

sisted of a gene pool restricted to wood bison and that bison at WBNP represented an integration of wood and plains bison gene pools. Given the results of the present study, this proposal now seems inappropriate. The presence of unique genotypes in these parks, however, suggests that consideration should be given to managing MBS, WBNP, and EINP (wood bison) populations apart from bison populations not containing the northern haplotypes and award them recognition as a unique population.

Genetic distances indicated that wood bison from WBNP and MBS were most related to each other ($d = 0.156$) and to wood bison at EINP ($d = 0.234$; Fig. 2). This is not surprising, since both MBS and EINP wood bison originated from a population in the same area of WBNP. Plains bison that historically share founding populations, however, do not show this trend. Bison at CSP and the NBR were the most similar to each other ($d = 0.140$) and, likewise, had the most haplotypes in the plains populations. Although the FNWR population shared founders with the NBR, it had the least number of haplotypes and did not cluster with other plains bison. Calculating the genetic distance from populations with few haplotypes, such as those at FNWR and YNP, places these populations together and more distant from other populations. However, taking small sample size, sampling error, and intrapopulation variability into consideration, the distance between all bison populations, a result of few founding members, is small and insignificant.

This study was performed to determine the genetic relationship of bison subspecies and their populations and not to estimate genetic diversity. However, based on distances between bison populations, the exchange of animals between parks to diversify the populations does not seem to be necessary. Genetic distances between bison populations are influenced by the limited number of founders and large number of shared founders. Including nuclear data would generate more genotypes but does not guarantee that distances would increase between populations. The unique haplotypes of the Custer State Park population did not set it apart from other populations. As a whole, bison vary little from one another and do not appear to suffer from inbreeding depression (Gates 1993), and the relocation of animals would raise other problems, such as introducing disease and placing stress on the animals.

Acknowledgments

We express our appreciation to the numerous park staff who collected our samples. The idea for PG-RFLP was suggested by John Sved. This work was supported by a Supply and Services Canada contract from the Canadian Parks Service and a Natural Sciences and Engineering Research Council of Canada grant (A0502) to C. Strobeck. Comments from our anonymous reviewers were also appreciated.

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Genetic variation within and relatedness among wood and plains bison populations

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Abstract: There are two recognized subspecies of bison, wood (*Bison bison athabascae*) and plains (*Bison bison bison*) bison. The establishment of most bison populations from a small number of individuals has raised concerns about their genetic variation. To this end, 11 bison populations were surveyed with 11 microsatellite loci in order to calculate genetic variation and genetic distances. Mean number of alleles ranged between 3.18 at Antelope Island State Park (Utah) and 6.55 at Wood Buffalo National Park (Alberta and Northwest Territories). Mean heterozygosity ranged from 0.295 at Antelope Island State Park to 0.669 at Custer State Park (South Dakota). The amount of genetic variability present in the bison populations as measured by mean number of alleles and overall probability of identity was found to correlate with the number of founders for all sampled populations. The *G*-test for heterogeneity revealed some evidence for the existence of subpopulations at Wood Buffalo National Park, however very small genetic distances between these subpopulations suggest that nuclear material from the plains bison introduced into Wood Buffalo National Park has diffused throughout the park. Genetic distances between the sampled populations were generally larger between than within the two bison subspecies.

Key words: *Bison bison bison*, *Bison bison athabascae*, DNA microsatellites, genetic variation, genetic relatedness.

Résumé : On reconnaît deux sous-espèces de bisons, le bison de forêt (*Bison bison athabascae*) et le bison des plaines (*Bison bison bison*). Le fait que la plupart des populations de bisons sont issues d'un petit nombre d'individus suscite des inquiétudes quant à la variation génétique au sein de celles-ci. Afin de mesurer cette variation et d'estimer des distances génétiques, onze populations de bison ont été étudiées à l'aide d'onze loci microsatellites. Le nombre moyen d'allèles variait entre 3,18 (Antelope Island State Park, Utah) et 6,55 (Wood Buffalo National Park, Alberta et Territoires du Nord-Ouest). Le degré moyen d'hétérozygotie allait de 0,295 (Antelope Island State Park, Utah) à 0,669 (Custer State Park, Dakota du Sud). La variabilité génétique présente chez les populations de bison, telle qu'estimée à l'aide du nombre moyen d'allèles et de la probabilité globale d'identité, s'est avérée corrélée avec le nombre d'individus fondateurs pour chacune des populations échantillonées. Le test de *G* pour l'hétérogénéité a montré des indices suggérant l'existence de sous-populations à Wood Buffalo National Park. Cependant, la très faible distance génétique entre ces sous-populations suggère que du matériel nucléaire provenant des bisons des plaines, lesquels ont été introduits à Wood Buffalo National Park, a diffusé partout dans le parc. Les distances génétiques entre les populations échantillonées étaient généralement plus grandes entre les deux sous-espèces qu'à l'intérieur de celles-ci.

Mots clés : *Bison bison bison*, *Bison bison athabascae*, microsatellites, variation génétique, degré de parenté génétique.

[Traduit par la Rédaction]

Introduction

Before the European settlement of North America, bison were among the most abundant local fauna. Herds of hundreds of animals roamed throughout most of North America. The amount of animal exchange between these herds is unknown, but has been suggested to be extensive (Roe 1970). Wood bison (*Bison bison athabascae*) existed in British Columbia, Alberta, and the Northwest Territories and plains bison (*Bison bison bison*) inhabited most of the remaining prairie regions. The amount of overlap of their ranges, if any, is unknown. Bison numbers were reduced to approximately 1000 by the late 1800s (Roe 1970), many of which were wood bison in what is now Wood Buffalo National Park (Alberta and Northwest Territories). The only other population containing indigenous animals is Yellowstone National Park (Wyoming), where between 22 and 50 animals existed in 1902 (Meagher 1973). The other remaining bison were found in a number of private herds throughout North America. Due to an intense restoration program by the governments of Canada and the United States, over 20 000 bison are currently found in public herds. However, the history of today's bison populations has raised concerns over the amount of genetic variability they contain.

Genetic variation is known to be greatly affected by the founder effect (Wright 1969; Nei et al. 1975). Populations originating from a small number of founders are expected to

Corresponding Editor: R.S. Singh.

Received August 28, 1998. Accepted October 30, 1998.

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contain less genetic variation than those started from a larger number. Most of the public bison populations were founded from few individuals. Bison used to found these herds were mainly from ranch herds started from few animals themselves, further decreasing the amount of genetic variation expected in these herds. In an attempt to decrease the effects of inbreeding (and increase genetic variation), some herds were founded from more than one bison strain. A lack of genetic variation has been linked to inbreeding effects in some populations (O'Brien et al. 1985). However, not all genetically depauperate populations are affected by inbreeding depression (see for example Paetkau and Strobeck 1994), and inbreeding does not seem to decrease fecundity in all bison populations (Berger and Cunningham 1995). The amount of genetic variation present in a number of wood and plains bison populations will be examined.

The history of bison populations also affects the relatedness among them. Populations started with animals from similar locations should be more genetically related than those with different strains. Therefore, one would expect to find larger genetic distances between wood and plains bison, especially if they are different subspecies.

However, the subspecific designation for wood bison is in doubt. Wood Buffalo National Park was created to protect the last remaining wood bison population. Unfortunately, a large herd of plains bison was moved to the Pine Lake region of Wood Buffalo National Park from 1925–1928, and the wood and plains bison in the park hybridized. A herd of what was thought to be pure wood bison was found in a secluded area of Wood Buffalo National Park, and animals were taken from this area to start herds of wood bison in Mackenzie Bison Sanctuary (Northwest Territories) and Elk Island National Park (Alberta) in 1963 and 1965, respectively. Since that time it has been accepted that the bison used to start these herds had hybridized, at least to some extent, with the plains bison (Van Zyll de Jong 1986). Geist (1991) has argued that this hybridization has led to the extinction of wood bison while Van Zyll de Jong et al. (1995) believe that wood bison are still different enough from plains bison to warrant subspecific status. If *Bison bison athabascae* does in fact exist, we would expect to find these populations more genetically differentiated from *Bison bison bison* than from each other.

There may be regions of Wood Buffalo National Park that contain bison which are close to pure wood bison, and other regions which are mostly plains bison. Van Camp (1989) stated that wood bison may still exist in isolated areas. Van Zyll de Jong et al. (1995) described the bison of the Sweetgrass region of Wood Buffalo National Park as the most morphologically similar to pure wood bison, and Pine Lake individuals as intermediate between wood and plains bison. For this to be true, the bison population at Wood Buffalo National Park would need to be fairly heterogeneous, with little gene flow between regions of the park. The heterogeneity of the Wood Buffalo National Park bison subpopulations can be measured to see if it is possible that pockets of mostly pure wood bison still exist. If the Wood Buffalo National Park population is homogeneous, no pure wood bison could exist in the park.

It has been proposed that the bison indigenous to Yellowstone National Park were actually a type of bison called mountain bison, referred to as *Bison bison athabascae* (Meagher 1973). Again, this taxonomic issue is in doubt (for review, see Roe 1970). Plains bison were also added to the indigenous herd at Yellowstone, which diluted the amount of local input to the gene pool to about 40% (Meagher 1973). If mountain bison did exist in this park, the current population should be genetically distinct from other bison populations which do not contain any mountain bison input in their gene pool, or more similar to wood bison as mountain bison and wood bison share the same subspecific designation.

Highly variable regions of the genome must be used to examine genetic variation, diversity, and heterogeneity. Bison contain little to no variation at the chromosomal and protein level (Ying and Peden 1977; Bork et al. 1991; Cronin and Cockett 1993; Stormont 1993). More polymorphism was detected by restriction digesting the control region of the mitochondrial DNA, but still some populations were monomorphic (Polziehn et al. 1996). DNA microsatellites are highly polymorphic nuclear markers (Tautz 1989; Weber and May 1989) and have been used to analyze the genetic relationships among populations (for review see Bruford and Wayne 1993), including those that are genetically depauperate (Hughes and Queller 1993; Paetkau et al. 1995). In this study the genetic variability, diversity, and heterogeneity in a number of public North American bison herds were investigated with 11 microsatellite loci.

Materials and methods

Laboratory methods

The populations used in this study were: plains bison from Antelope Island State Park (Utah, AISP), Custer State Park (South Dakota, CSP), Elk Island National Park (Alberta, EINPP), Fort Niobrara National Wildlife Refuge (Nebraska, FNWR), National Bison Range (Montana, NBR), Wichita Mountains Wildlife Refuge (Oklahoma, WMWR), and Yellowstone National Park (Wyoming, YNP); wood bison from Elk Island National Park (EINPW), Mackenzie Bison Sanctuary (Northwest Territories, MBS), and Wood Buffalo National Park (Alberta and Northwest Territories, WBNP); and a feral herd of plains bison from Pink Mountain (British Columbia, PM). Table 1 summarizes the origins of these herds. EINPP had 45 founders as all other bison were shipped from the park to Buffalo National Park in 1909. While 18 and 24 animals were shipped from WBNP to MBS and EINPW, respectively, only 16 survived the trip to MBS. All of the adults were destroyed in EINPW to eradicate brucellosis, leaving 11 animals as founders (C. Gates, personal communication). To test the heterogeneity of the Wood Buffalo National Park population, samples from that park were split into the subpopulations of Garden River (GR), Little Buffalo (LB), Needle Lake (NL), Pine Lake (PL), and Sweetgrass (SW).

Sample sizes from the populations were: 30 from AISP, 32 from CSP, 30 from EINPP, 30 from FNWR, 30 from NBR, 21 from WMWR, 33 from YNP, 36 from EINPW, 28 from MBS, and 81 from WBNP. Of the WBNP samples, 8 were from GR, 13 from LB, 14 from NL, 24 from PL, and 22 from SW. DNA samples from AISP were kindly supplied by Julie Schneider. Tissue samples from PM, and DNA from all other populations were obtained from the DNA repository maintained by the Canadian Parks Service at the University of Alberta. As bison groups are quite fluid, and associations between individuals random, it can be assumed that these are random samples from the populations (Lott and

Table 1. Origins of the bison herds used in this study (Garretson 1938; Rorabacher 1970; Meagher 1973; Dary 1974; Coder 1975; Ogilvie 1979; Jenning and Hebbing 1983; Christiansen 1991; Malcolm 1993; Polziehn 1993; C. Gates, personal communication; R. Walker, personal communication; and T. Novak, personal communication).

Owner	Origin	Number	Year
Ranch herds			
Alloway/McKay	Saskatchewan	5	1873–4
Bedson	Manitoba (?)	3	1880
Bedson	Alloway/McKay	8	1880
Strathcona	Alloway/McKay	all	1887
Goodnight	Texas panhandle	6	1878
Goodnight	a Texas ranch (origin unknown)	1	1878
Goodnight	death, before breeding	–2	1878
Walking Coyote	Montana	4	1879
Pablo/Allard	Walking Coyote	12	1883
Pablo/Allard	Jones	26	1893
Conrad	Pablo/Allard	30	1902
Eaton	Pablo/Allard	60	1902
Dupree	Montana	6–7 (?)	1882
McCoy	Oklahoma (?)	2	1882
PWFC	McCoy	2	1886
Jones	Oklahoma	56	1886–9
Jones	Bedson	all (~75)	1889
Jones	Kansas, Nebraska ranches	10	1889
Corbin	Jones (From Bedson)	12	1889
Corbin	Jones	10	1892
Corbin	Banff NP	2	1904
Whitney	Wyoming (?)	13	1897
Whitney	Jones (from Oklahoma)	1	1897
Philip	Dupree	~75	1901–2
Gilbert	PWFC	3	1902
Gilbert	ranch in Iowa	1	1903
Public Herds			
AISP	Jones (from Bedson?)	12	1893
NYZG	Goodnight	4	1899
NYZG	Oklahoma	3	1899
NYZG	?	3	1900
NYZG	Wyoming	13	1903
NYZG	PWFC	1M, 3F	1904
NYZG	Whitney	13 (?)	1900–04
NYZG	Whitney (Oklahoma Jones animal)	1	1901
Banff NP	Goodnight	3	1897
Banff NP	Strathcona	13	1898
Banff NP	Corbin	2M	1904
YNP	native	22–50	1902
YNP	Eaton	18F	1902
YNP	Goodnight	3M	1902
WMWR	NYZG	6M, 9F	1907
WMWR	FNWR	4M	1942
WMWR	NBR	4M	1952
EINPP	Pablo/Allard	183	1907
EINPP	Banff NP	7M	1907
BNP	EINPP	all but 45	1909
Banff NP	Pablo/Allard	16M	1907
BNP	Pablo/Allard	298	1909–12
BNP	Banff NP	91	1909–14
NBR	Conrad	37	1909
NBR	Goodnight, Corbin, Jones	12	1910–11
NBR	WMWR	4M	1952

Table 1. (*concluded*).

Owner	Origin	Number	Year
NBR	YNP	2M	1953
WCNP	NYZG	7M, 7F	1914
FNWR	Gilbert	6	1913
FNWR	YNP	2M	1913
FNWR	CSP	8	1935–7
FNWR	NBR	5	1952
CSP	Philip	36	1914
CSP	Pine Ridge Reservation (origin unknown)	few	1940s
CSP	WCNP	~800	1950s
MBS	WBNP	16	1963
WBNP	native	200 minimum	
WBNP	BNP	6673	1925–28
EINPW	WBNP	11	1965
PM	EINPP	48	1971

Note: abbreviations: Page Woven Fence Company (PWFC), Antelope Island State Park (AISP), New York Zoological Gardens (NYZG), Yellowstone National Park (YNP), Wichita Mountains Wildlife Refuge (WMWR), Fort Niobrara Wildlife Refuge (FNWR), National Bison Range (NBR), plains bison at Elk Island National Park (EINPP), Buffalo National Park (Wainwright, BNP), Wind Cave National Park (WCNP), Custer State Park (CSP), Mackenzie Bison Sanctuary (MBS), wood bison at Elk Island National Park (EINPW), and Pink Mountain (PM).

Table 2. Remainder of the contents and amounts for the PCR reactions. Loci 1A, 1B, and 1C are *BM143*, *BM2830*, and *BM1225*, respectively. *RT29*, *BMC1222*, and *BM4513* are Primers A, B, and C in reaction mix 2, respectively. *RT27*, *RT24*, and *RT9* are multiplexed as loci 3A, 3B, and 3C, respectively. *Eth121* and *BOVFSH* are amplified separately using reaction mix 4.

	Reaction Mix 1 (μM)	Reaction Mix 2 (μM)	Reaction Mix 3 (μM)	Reaction Mix 4 (μM)
Primers A	0.19	0.19	0.18	0.16
Primers B	0.19	0.19	0.16	–
Primers C	0.17	0.13	0.16	–
dNTPs	160	160	160	120
Taq polymerase	0.68 units	0.48 units	0.6 units	0.6 units

Minta 1983; Van Vuren 1983). DNA was extracted from the PM tissue samples using a QIAamp® Tissue Extraction Kit.

The microsatellite loci used in this study were: *BM143*, *BM1225*, *BM2830*, *BM4513*, and *BMC1222* from Bishop et al. (1994), *BOVFSH* from Moore et al. (1992), *Eth121* from Steffen et al. (1993), and *RT9*, *RT24*, and *RT27* from Wilson et al. (1997). *RT29*, also used in the study, is *RT1* from Wilson et al. (1997) modified so that the primer sequences are GCCTTCTTTCATCCAACAAA and CCCATCTTCCCATCCTCTT. A FAM, HEX, or TET fluorescent dye group was added to the 5′ end of one primer from each of these loci. Where possible, these loci were multiplexed during PCR. Multiplexing in this context refers to the amplification of more than one locus in a single PCR reaction. All PCR reactions contained 2.5 mM $MgCl_2$ and approximately 60 ng DNA. The remainder of the PCR reaction contents, and multiplexes, are given in Table 2. The PCR was done on an ABI 9600 thermal cycler. Cycling conditions for the PCR reactions were as follows: 1 min at 94°C; then three cycles of 30 s at 94°C, 20 s at 54°C, and 5 s at 72°C; then 33 cycles of 15 s at 94°C, 20 s at 54°C, and 1 s at 72°C; then 30 s at 72°C. The PCR amplifications were then visualized with an ABI 373A DNA Sequencer and GENESCAN 672 software.

Data analysis

To examine variability in the bison populations, we first determined the allele frequencies of each locus for the populations. Allele frequencies are the prevalence of each type of allele in a population. We then used this information to calculate mean number of alleles, average heterozygosity, and overall probability of identity (*pI*). Mean number of alleles is the average number of alleles a population has present at any given locus. Average heterozygosity is the expected number of individuals having copies of different alleles at any locus. Unbiased expected heterozygosity was calculated at each locus using the formula from Nei and Roychoudhury (1974). This was then averaged over all loci to obtain the mean heterozygosity for each population. Probability of identity is the probability that an individual's genotype will be identical to another individual's genotype in the population, and can be calculated with formula [1]

$$[1] \qquad \sum_i p_i^4 + \sum_i \sum_{j>i} (2p_i p_j)^2$$

where p_i and p_j are the frequencies of the i^{th} and j^{th} alleles, respectively. We obtained unbiased probability of identity values using a formula from Paetkau et al. (1998). The overall *pI* for a population was calculated by multiplying together the probabilities of identity for all surveyed loci in that population. Three methods of measuring the original size of the bison populations were compared to these three measures of genetic variation using Kendall's rank correlation test (Sokal and Rohlf 1995). The three measures of original population size were defined as: (*i*) park founders, the number of animals originally used to found each of the herds, (*ii*) number in original stock, this value is similar to park founders but cannot exceed the lowest number of founders from the ranch populations,

Table 3. Allele frequencies, heterozygosities (*H*), and probabilities of identity (*pI*) for each population at each locus. The sample size (*n*) is given in the first table. If known, the size of the loci in cattle (CSize) is also given. Abbreviations can be found in Table 1.

Table 3A. Locus *BM143* (CSize 90–122).

		Allele frequencies									
Population	*n*	99	101	103	105	109	111	113	115	*H*	*pI*
AISP	30	0.017	0.75		0.167	0.033			0.033	0.414	0.37
CSP	32	0.094	0.094	0.109	0.281		0.172	0.031	0.219	0.826	0.053
EINPP	30		0.25	0.1	0.167		0.15		0.333	0.779	0.083
FNWR	30	0.117	0.567	0.083	0.117				0.117	0.642	0.154
NBR	30		0.533		0.2			0.033	0.233	0.631	0.192
PM	19		0.184	0.342	0.132		0.053		0.289	0.765	0.094
WMWR	21		0.286	0.095	0.238		0.381			0.725	0.128
YNP	33		0.212	0.242	0.076		0.333	0.045	0.091	0.781	0.081
EINPW	36	0.014	0.472		0.514					0.52	0.351
MBS	28	0.375	0.339	0.018	0.089	0.071	0.018		0.089	0.736	0.11
WBNP	81	0.179	0.506	0.049	0.167	0.031	0.025		0.043	0.682	0.135

Table 3B. Locus *BM2830* (CSize 149–203).

	Allele frequencies													
Population	141	143	147	149	151	153	155	157	159	163	165	167	*H*	*pI*
AISP			0.25	0.4	0.083				0.067	0.183		0.017	0.745	0.103
CSP			0.156	0.109	0.031	0.234	0.047	0.063	0.297	0.047	0.016		0.824	0.052
EINPP		0.017			0.133	0.083		0.067	0.417	0.25	0.033		0.746	0.098
FNWR			0.117	0.017		0.233		0.033	0.35	0.05	0.2		0.779	0.081
NBR			0.067	0.15	0.017	0.183			0.117	0.4	0.067		0.774	0.077
PM				0.053	0.132	0.184			0.421	0.211			0.744	0.101
WMWR			0.048	0.095	0.238	0.405		0.024		0.19			0.749	0.099
YNP	0.091		0.015	0.045	0.061	0.303			0.212	0.045	0.227		0.807	0.063
EINPW			0.25			0.583	0.139					0.028	0.585	0.228
MBS			0.321			0.5			0.054		0.036	0.089	0.646	0.183
WBNP		0.012	0.216	0.025	0.019	0.481		0.012	0.123	0.037	0.025	0.049	0.705	0.119

and (*iii*) number of strains, the number of different origins for the bison used to found the park herds.

The Monte Carlo approximation of the Fisher's exact test was used to detect deviations from Hardy-Weinberg equilibrium (Guo and Thompson 1992). Loci displaying an excess of homozygotes may contain null alleles, known to be present in a number of loci (Callen et al. 1993; Koorey et al. 1993; Paetkau and Strobeck 1995). Allele distributions were compared between populations using a *G*-test for heterogeneity (Sokal and Rohlf 1995). The *G*-test was chosen as it makes no assumptions about the method of mutation through which microsatellite alleles are derived. Pairwise comparisons between all populations at all loci were performed, and summed over all loci. The *G*-test was also used to examine the heterogeneity of the WBNP subpopulations. The assignment test, which compares an individual's genotype to the allele frequencies in all populations and assigns it to the population most likely to contain the genotype, was also calculated (Paetkau et al. 1995).

Statistical measures based on the infinite allele model (IAM), as opposed to the stepwise mutation model (SMM), were stressed as they give more reliable results with microsatellite data (Takezaki and Nei 1996; Paetkau et al. 1997). To obtain the genetic relatedness of the bison populations, Nei's standard (D_S, Nei 1972), Nei's minimum (D_M, Nei 1973), delta-mu squared ($\delta\mu^2$, Goldstein et al. 1995), and the genotype likelihood ratio (D_{LR}, Paetkau et al. 1997) genetic distance methods were calculated between all population pairs, and the subpopulations at WBNP. These measures were chosen because D_S and D_M are popular IAM methods of computing genetic distance. $(\delta\mu)^2$, based on the SMM, was designed specifically for microsatellites. D_{LR} is based on the assignment test. It is the log likelihood of a genotype occurring in a population other than its parent population. For example, a D_{LR} value of two means that genotypes are two orders of magnitude more likely to occur in the parent population than the other population being compared. Programs to calculate all of these genetic distances were designed by John Brzustowski and are available at http: //www.biology.ualberta.ca/jbrzusto/GeneDist.html. Unrooted trees from the population genetic distance data were created by PHYLIP 3.572 (Felsenstein 1995), using the neighbour-joining (Saitou and Nei 1987) and Fitch and Margoliash (1967) methods.

Results

Allele frequencies for all of the sampled populations at all loci, and their corresponding heterozygosities and probabilities of identity are given in Table 3. A range of variation was seen in the sampled loci. Average number of alleles was highest at locus *BOVFSH* and lowest at *BM4513* with values of 9.18 and 1.36, respectively. Locus *RT29* had the highest average heterozygosity with 0.767 and *BM4513* the lowest with 0.078. The lowest *pI* occurred for locus *RT29*, with a value of 0.092, and the highest at *BM4513*, with a value of

Table 3C. Locus *BM4513* (CSize 141–161).

Population	Allele frequencies 133	135	*H*	*pI*
AISP	0.917	0.083	0.155	0.72
CSP	0.813	0.188	0.31	0.519
EINPP	1		0	1
FNWR	0.917	0.083	0.155	0.72
NBR	1		0	1
PM	1		0	1
WMWR	1		0	1
YNP	0.864	0.136	0.239	0.601
EINPW	1		0	1
MBS	1		0	1
WBNP	1		0	1

Table 3D. Locus *BMC1222* (CSize 272–302).

Population	Allele frequencies 267	273	275	277	*H*	*pI*
AISP			1		0	1
CSP	0.094	0.156	0.656	0.094	0.536	0.246
EINPP	0.3		0.7		0.427	0.416
FNWR	0.183	0.05	0.767		0.382	0.418
NBR	0.15	0.033	0.717	0.1	0.46	0.318
PM	0.132		0.868		0.235	0.602
WMWR	0.024	0.024	0.952		0.094	0.816
YNP	0.03		0.97		0.06	0.883
EINPW		0.097	0.444	0.458	0.591	0.258
MBS	0.036	0.054	0.893	0.018	0.202	0.636
WBNP	0.043	0.123	0.525	0.309	0.616	0.212

Table 3E. Locus *BM1225* (CSize 227–253).

Population	Allele frequencies 238	240	244	246	248	252	264	268	270	272	*H*	*pI*
AISP		0.717				0.15	0.133				0.454	0.334
CSP	0.141	0.344			0.063	0.234	0.109	0.016	0.094		0.795	0.069
EINPP	0.017	0.467	0.15	0.017	0.083	0.15		0.033	0.083		0.734	0.095
FNWR	0.1	0.633				0.067	0.017	0.167		0.017	0.566	0.217
NBR	0.017	0.383		0.033	0.033	0.117		0.317	0.017	0.083	0.742	0.105
PM		0.368	0.211	0.026	0.053	0.079		0.079	0.184		0.791	0.07
WMWR		0.738				0.167		0.024	0.071		0.432	0.348
YNP		0.5				0.152		0.091	0.258		0.662	0.164
EINPW		0.042			0.111	0.583		0.264			0.584	0.229
MBS		0.196				0.643		0.161			0.532	0.269
WBNP		0.179	0.006	0.093	0.006	0.438	0.006	0.222	0.037	0.012	0.721	0.117

Table 3F. Locus *BOVFSH* (CSize 291–320).

Population	Allele frequencies 296	298	299	302	303	304	308	309	310	311	312	313	316	317	321	322	325	*H*	*pI*
AISP	0.017							0.983										0.033	0.933
CSP	0.125	0.125		0.016	0.328	0.047	0.016	0.109				0.125	0.047		0.063			0.838	0.041
EINPP	0.067	0.05		0.05	0.017		0.167	0.2	0.1			0.05			0.25	0.05		0.859	0.035
FNWR	0.167	0.383			0.017					0.167		0.05	0.05		0.1		0.067	0.791	0.066
NBR		0.017		0.683	0.05	0.017	0.017					0.017	0.017		0.183			0.504	0.274
PM		0.105		0.026	0.105		0.211	0.158	0.105			0.053			0.132	0.105		0.889	0.023
WMWR	0.048	0.024		0.095		0.071	0.048					0.429	0.286					0.733	0.106
YNP	0.015	0.152		0.03	0.106	0.182	0.015	0.273		0.136		0.015		0.015	0.03	0.03		0.849	0.039
EINPW			0.056	0.014	0.083			0.528		0.125		0.014		0.042	0.139			0.684	0.122
MBS		0.018	0.036	0.036	0.304		0.018	0.179		0.161		0.036		0.018	0.143	0.054		0.837	0.044
WBNP	0.025	0.025	0.031	0.062	0.123		0.049	0.333	0.012	0.136	0.006	0.043	0.012	0.037	0.093	0.012		0.84	0.039

0.869. Some alleles were fixed in various bison populations. Allele 133 at locus *BM4513* was fixed in populations EINPP, NBR, PM, WMWR, EINPW, MBS, and WBNP. Allele 275 at locus *BMC1222* was fixed in AISP. Alleles unique to a population were also discovered. At locus *BOVFSH*, allele 312 was found only in the WBNP population, and allele 325 was present only in FNWR individuals. YNP had two unique alleles, 209 and 211, at locus RT24.

As each locus for each population must be checked for Hardy-Weinberg equilibrium, there were a total of 121 genotype distributions to be examined using the Monte-Carlo approximation of the Fisher's exact test. However, eight of the allele distributions were monomorphic, so only 113 tests of the Hardy-Weinberg distributions were calculated. Eight of the genotype distributions were outside of the Hardy-Weinberg expectations at the 10% level and 5 at the 5%

Table 3G. Locus *Eth121* (CSize 173–212).

Population	Allele frequencies					*H*	*pI*
	186	188	194	198	200		
AISP	0.8	0.133		0.017	0.05	0.345	0.447
CSP	0.375	0.453	0.031	0.094	0.047	0.652	0.185
EINPP	0.433	0.1	0.05		0.417	0.637	0.205
FNWR	0.183	0.383	0.283	0.117	0.033	0.737	0.113
NBR	0.55	0.35	0.017		0.083	0.577	0.258
PM	0.237	0.079		0.079	0.605	0.58	0.22
WMWR	0.762	0.071		0.167		0.396	0.393
YNP	0.788	0.03	0.015	0.061	0.106	0.369	0.41
EINPW	0.403			0.111	0.486	0.597	0.249
MBS	0.911				0.089	0.166	0.704
WBNP	0.469	0.049	0.037	0.068	0.377	0.634	0.202

Table 3H. Locus *RT9*.

Population	Allele frequencies				*H*	*pI*
	113	115	117	119		
AISP	0.95	0.05			0.097	0.817
CSP	0.516	0.141	0.344		0.606	0.233
EINPP	0.617	0.067	0.317		0.524	0.306
FNWR	0.75		0.167	0.083	0.41	0.381
NBR	0.783	0.117	0.083	0.017	0.372	0.412
PM	0.316	0.158	0.526		0.615	0.22
WMWR	0.405	0.143	0.405	0.048	0.666	0.179
YNP	0.394	0.152	0.455		0.625	0.221
EINPW	0.694	0.056	0.25		0.459	0.354
MBS	0.786	0.089	0.125		0.366	0.423
WBNP	0.858	0.043	0.099		0.254	0.571

Table 3I. Locus *RT24*.

Population	Allele frequencies								*H*	*pI*
	205	209	211	213	225	227	229	231		
AISP	0.967						0.017	0.017	0.066	0.87
CSP	0.656				0.109		0.188	0.047	0.528	0.26
EINPP	0.7			0.05	0.15		0.05	0.05	0.488	0.283
FNWR	0.583				0.383	0.017		0.017	0.521	0.331
NBR	0.5				0.133	0.283		0.083	0.656	0.172
PM	0.868			0.026	0.053		0.053		0.246	0.564
WMWR	0.857				0.071		0.071		0.261	0.549
YNP	0.742	0.015	0.015	0.045	0.03	0.106		0.045	0.439	0.325
EINPW	0.625						0.306	0.069	0.518	0.308
MBS	0.661						0.339		0.456	0.397
WBNP	0.685			0.012	0.025	0.006	0.228	0.043	0.479	0.322

level. This number of values out of Hardy-Weinberg equilibrium is expected considering the number of tests performed. When the Dunn-Sidák experiment-wise error rate was used (Sokal and Rohlf 1995), two genotype distributions deviated from Hardy-Weinberg equilibrium at the 5% level, one of which also deviated at the 1% level. This was the *Eth121* allele frequency for the AISP population. A chi-squared goodness-of-fit test showed the observed number of heterozygotes for this genotype distribution did not deviate from the expected value at the 5% level. The deviation from Monte-Carlo expectations seems to be due to an inordinate number of 188/200 heterozygotes.

Table 4 shows the mean number of alleles, average heterozygosities and overall *pI* for all populations. The AISP population was the least variable with all three measures. The WBNP population was most variable when examining the mean number of alleles, while CSP displayed the most variation with the other two measures. All three methods of measuring variation showed that MBS and EINPW were both notably less variable than their founder population at WBNP. PM was also less variable than its founder population, EINPP, using all three methods of measuring variation.

The rankings used in the correlation analyses are given in Table 5. Rankings were calculated using the values from Table 1, but a few assumptions were made. (*i*) As CSP had 800 animals added to it from Wind Cave National Park, the number of founders for this park were added to the CSP population. (*ii*) For the rankings of the number of animals in the original stock, if the animals were moved more than once before reaching their final destination of the public herd, the smallest number of founders from the moves was considered. For example, in 1914, CSP was founded from 36 animals from Philip, who in turn had started his herd from about 70 Dupree animals. As Dupree had started his herd

Table 3J. Locus *RT27*.

Population	Allele frequencies				*H*	*pI*
	146	148	150	152		
AISP	0.133		0.867		0.235	0.606
CSP	0.422	0.031	0.453	0.094	0.617	0.227
EINPP	0.033	0.033	0.933		0.129	0.757
FNWR	0.4		0.517	0.083	0.576	0.272
NBR	0.2	0.1	0.65	0.05	0.534	0.257
PM	0.026		0.974		0.053	0.895
WMWR	0.214	0.048	0.738		0.417	0.385
YNP	0.091	0.091	0.818		0.319	0.479
EINPW	0.333		0.667		0.451	0.401
MBS	0.089		0.911		0.166	0.704
WBNP	0.173	0.012	0.802	0.012	0.328	0.488

Table 3K. Locus *RT29*.

Population	Allele frequences								*H*	*pI*
	206	212	214	216	218	220	222	224		
AISP	0.233	0.033			0.267	0.433	0.033		0.696	0.147
CSP	0.219	0.234	0.188		0.125	0.156	0.031	0.047	0.832	0.051
EINPP	0.083	0.267	0.117	0.05	0.133	0.133		0.217	0.837	0.047
FNWR	0.117	0.15	0.267		0.417		0.05		0.729	0.115
NBR		0.083	0.067		0.317	0.383	0.033	0.117	0.739	0.108
PM	0.053	0.395	0.053		0.158	0.263		0.079	0.758	0.092
WMWR	0.381				0.024	0.167	0.286	0.143	0.742	0.11
YNP	0.106	0.045	0.03		0.106	0.227	0.197	0.288	0.814	0.061
EINPW	0.25	0.125	0.014			0.167	0.417	0.028	0.73	0.114
MBS	0.036	0.393	0.018		0.018	0.268	0.179	0.089	0.745	0.103
WBNP	0.259	0.198	0.056	0.012	0.056	0.235	0.16	0.025	0.811	0.063

Table 4. Mean values for number of alleles, mean heterozygosity, and overall probability of identity (*pI*), the product of the *pI* values at each locus, for the bison populations. Abbreviations are given in Table 1.

	Mean no. alleles	Mean heterozygosity	1 / overall *pI*
AISP	3.18	0.295	4 100
CSP	5.64	0.669	7 600 000 000
EINPP	5.00	0.560	140 000 000
FNWR	4.64	0.572	42 000 000
NBR	4.91	0.544	15 000 000
PM	4.36	0.516	48 000 000
WMWR	3.91	0.474	1 600 000
YNP	5.36	0.542	67 000 000
EINPW	3.64	0.520	1 400 000
MBS	4.27	0.441	760 000
WBNP	6.55	0.552	57 000 000

from seven animals, the number of original animals starting the CSP population was then considered to be seven (excluding those from Wind Cave National Park). (*iii*) The number of strains in a herd was calculated by counting the number of strains in each addition of bison. For example, Mackenzie Bison Sanctuary, founded solely from animals from Wood Buffalo National Park, was assigned the same number of strains as the latter park. The number of strains could not exceed the number of bison added. (*iv*) If the number of animals added to a population was less than the number of founders for the emigrant population, the number of founders was added to the immigrant population instead of the actual number of individuals added. (*v*) Additions of less than eight animals to a public herd more than 20 y after it was originally founded were ignored. (*vi*) It was assumed that PM could not have a larger founding size than EINPP, as it was started solely from the latter population, so they were assigned a tie in the rankings. (*vii*) It was assumed that YNP had 50 native bison in the park in 1902 (Meagher 1973). And (*viii*) WMWR is known to have been founded from six strains of bison from the New York Zoological Gardens (Coder 1975). As Wind Cave National Park was also founded from New York Zoological Gardens, it was also assumed to have six strains of bison. The number of founders correlated with the mean number of alleles ($P < 0.01$) and the overall probability of identity ($P < 0.1$), while the number in original stock correlated with the mean num-

Table 5. Rankings assigned to the sampled bison populations used in Kendall's rank correlation test. The first value is the ranking, the second number in parenthesis is the actual value for the size of founding population measures. Actual values for the variability measures are in Table 4. Abbreviations are given in Table 1.

Table 5A. Size of founding population measures.

Park founders		Number of strains		Number in original stock	
FNWR	1 (8)	AISP	1 (2)	FNWR	1 (5)
EINPW	2 (11)	FNWR	2 (4)	AISP	2 (8)
AISP	3 (12)	WMWR	3 (6)	EINPW	3 (11)
WMWR	4 (15)	NBR	6 (8)	WMWR	4 (15)
MBS	5 (16)	YNP	6 (8)	MBS	5 (16)
EINPP	6.5 (45)	EINPP	6 (8)	CSP	6 (26)
PM	6.5 (45)	PM	6 (8)	PM	7.5 (37)
NBR	8 (49)	CSP	6 (8)	EINPP	7.5 (37)
CSP	9 (55)	WBNP	10 (9)	NBR	9 (42)
YNP	10 (71)	EINPW	10 (9)	YNP	10 (71)
WBNP	11 (240)	MBS	10 (9)	WBNP	11 (240)

Table 5B. Variability measures.

Mean no. alleles		Heterozygosity		Overall probability of identity	
AISP	1	AISP	1	AISP	1
EINPW	2	MBS	2	MBS	2
WMWR	3	WMWR	3	EINPW	3
MBS	4	PM	4	WMWR	4
PM	5	EINPW	5	NBR	5
FNWR	6	YNP	6	FNWR	6
NBR	7	NBR	7	PM	7
EINPP	8	WBNP	8	WBNP	8
YNP	9	EINPP	9	YNP	9
CSP	10	FNWR	10	EINPP	10
WBNP	11	CSP	11	CSP	11

Table 6. Assignment test results. Individuals assigned to their source population are in bold. Individuals assigned to the incorrect subspecies are in italics. Abbreviations can be found in Table 1.

	Population to which the individual was assigned										
Source	AISP	CSP	EINPP	FNWR	NBR	PM	WMWR	YNP	EINPW	MBS	WBNP
AISP	**30**										
CSP		**30**		**1**	**1**						
EINPP	**1**		**17**	**1**	**1**	**10**					
FNWR				**30**							
NBR	**1**	**1**			**28**						
PM			**6**			**12**		**1**			
WMWR							**21**				
YNP			**1**			**1**	**2**	**29**			
EINPW									**33**	**2**	**1**
MBS										**27**	**1**
WBNP	*1*	*1*		*1*		*2*			**17**	**10**	**49**

Table 7. Results of the assignment test for the Wood Buffalo National Park individuals, sorted by subpopulation. The total number of individuals in each subpopulation is in the first column (Total). The number of animals from each subpopulation assigned to WBNP, and all other populations, are in the other columns. Populations not listed had no WBNP animals assigned to them. Abbreviations for the populations can be found in Table 1.

					Plains bison populations			
	Total	WBNP	EINPW	MBS	AISP	CSP	FNWR	PM
GR	**8**	6	1	1				
LB	**13**	8	2	2		1		
NL	**14**	8	5	1				
PL	**24**	12	7	4				1
SW	**22**	15	2	2	1		1	1

Note: abbreviations for the subpopulations: Garden River (GR), Little Buffalo (LB), Needle Lake (NL), Pine Lake (PL), and Sweetgrass (SW).

ber of alleles ($P < 0.01$). None of the variability measures correlated with the number of strains per herd.

All pairs of populations had significantly different allele distributions when using the G-test ($P < 0.001$). However, of the WBNP subpopulations, only the allele distributions for the NL–GR, PL–GR, PL–LB, PL–NL, PL–SW, and NL–SW comparisons differed significantly at the 10% level using the G-test. Only NL–PL and NL–SW were significantly different when $P < 0.001$.

Of the 370 individuals used in the assignment test, 276 of them (75%) were assigned to the correct population (Table 6). Only five (1.4%) of the incorrectly assigned animals were placed in the incorrect subspecies, and all of these were WBNP bison assigned to various plains bison populations. Thirty-three percent of the WBNP animals were assigned to either EINPW or MBS. Thirty-three percent of the individuals from EINPP were specifically assigned to PM and 32% of the PM bison were assigned to EINPP. FNWR, AISP, and WMWR each had all of their individuals assigned to the correct population. Table 7 shows the number of individuals from each of the WBNP subpopulations that were assigned to the various populations. Of the five WBNP animals assigned to plains bison populations, three were from SW, one was from PL and one was from LB.

The genetic distances between all population pairs, and the WBNP subpopulations, can be found in Table 8. For D_S, the smallest distances between populations were the EINPP–PM and the WBNP–EINPW distances. The largest between population distance was between EINPW and FNWR. The smallest interpopulation distance with the D_M measure was between both WBNP–EINPW and EINPP–PM. The largest D_M distances were EINPW–AISP and AISP–PM. The D_{LR} genetic distance measure had the smallest interpopulation distance when comparing the EINPP and PM populations and the largest when measuring the EINPW–FNWR distance. $(\delta\mu)^2$ had the smallest interpopulation values when measuring the WBNP–EINPW distance and the largest when measuring the EINPW–AISP and NBR–AISP distances.

Unrooted trees for the populations were designed for all four of these genetic distance measures by PHYLIP 3.572, using the neighbour-joining (Saitou and Nei 1987) and Fitch and Margoliash (1967) methods. The unrooted tree made by applying the Fitch and Margoliash algorithm to the D_M distances is shown in Fig. 1. Aside from minor differences in branch lengths, both the neighbour-joining and Fitch and Margoliash algorithms gave trees identical to this one for the D_S, D_M, and D_{LR} distance measures, except the D_{LR} neighbour-joining tree has the (FNWR–CSP–NBR) and (WMWR–YNP) branches exchanged. Wood bison form one group on this tree. The unrooted tree created using $(\delta\mu)^2$ was not analyzed, as it was quite different from the other three. The $(\delta\mu)^2$ measure has been found to have high variance, and this could be the reason that its results differ from the other three (Paetkau et al. 1997). Takezaki and Nei (1996)

Table 8. Genetic distances between all bison populations, and the subpopulations at Wood Buffalo National Park (in bold). Distances between Wood Buffalo National Park and its subpopulations were not calculated. Population abbreviations can be found in Table 1, and subpopulation abbreviations are in Table 7.

Table 8A. D_S results are above the diagonal and D_M are below the diagonal.

	AISP	CSP	EINPP	FNWR	NBR	PM	WMWR	YNP	EINPW	MBS	WBNP	GR	LB	NL	PL	SW
AISP		0.261	0.204	0.261	0.215	0.290	0.217	0.184	0.280	0.209	0.171	0.239	0.201	0.221	0.155	0.179
CSP	0.147		0.162	0.151	0.225	0.192	0.172	0.168	0.257	0.195	0.181	0.224	0.205	0.237	0.185	0.177
EINPP	0.119	0.062		0.190	0.174	0.053	0.182	0.126	0.279	0.193	0.155	0.191	0.182	0.258	0.149	0.133
FNWR	0.145	0.057	0.077		0.194	0.272	0.258	0.251	0.377	0.302	0.231	0.253	0.236	0.292	0.266	0.209
NBR	0.124	0.085	0.073	0.08		0.269	0.240	0.230	0.320	0.226	0.19	0.208	0.211	0.255	0.195	0.187
PM	0.159	0.079	0.025	0.112	0.114		0.218	0.141	0.320	0.251	0.21	0.236	0.244	0.341	0.189	0.188
WMWR	0.126	0.079	0.084	0.113	0.108	0.102		0.090	0.303	0.204	0.218	0.246	0.282	0.289	0.199	0.212
YNP	0.109	0.067	0.054	0.100	0.095	0.064	0.044		0.296	0.159	0.196	0.253	0.260	0.282	0.163	0.185
EINPW	0.154	0.099	0.114	0.146	0.130	0.135	0.135	0.122		0.163	0.055	0.078	0.070	0.053	0.069	0.088
MBS	0.123	0.092	0.092	0.134	0.106	0.119	0.102	0.078	0.081		0.074	0.116	0.099	0.107	0.078	0.084
WBNP	0.104	0.069	0.065	0.092	0.079	0.091	0.098	0.082	0.025	0.081						
GR	0.135	0.085	0.080	0.101	0.087	0.102	0.110	0.104	0.036	0.059			0.038	0.063	0.045	0.043
LB	0.118	0.081	0.077	0.097	0.090	0.106	0.125	0.108	0.033	0.051		0.017		0.036	0.045	0.036
NL	0.128	0.095	0.109	0.120	0.120	0.144	0.131	0.119	0.026	0.055		0.030	0.017		0.061	0.063
PL	0.096	0.070	0.062	0.104	0.081	0.082	0.091	0.069	0.032	0.041		0.020	0.021	0.029		0.035
SW	0.107	0.069	0.057	0.085	0.079	0.083	0.097	0.079	0.040	0.044		0.020	0.017	0.030	0.016	

Table 8B. D_{LR} results are above the diagonal and $(\delta\mu)^2$ results are below the diagonal.

	AISP	CSP	EINPP	FNWR	NBR	PM	WMWR	YNP	EINPW	MBS	WBNP	GR	LB	NL	PL	SW
AISP		6.63	6.5	8.73	6.85	7.42	7.22	6.29	8.23	6.59	5.41	6.14	5.15	6.43	4.99	5.15
CSP	10.77		3.91	3.61	5.47	4.25	4.73	4.03	6.58	5.06	3.80	3.93	3.42	5.06	4.02	3.71
EINPP	12.43	7.27		5.14	4.25	0.46	5.89	3.46	8.32	5.85	4.55	4.54	4.57	6.75	4.48	3.61
FNWR	10.57	3.70	6.10		5.57	6.58	7.42	6.26	10.38	7.79	5.83	5.43	5.11	7.58	6.89	4.97
NBR	21.70	6.80	12.69	8.80		5.59	6.53	5.54	8.73	6.52	5.11	4.48	4.62	7.07	5.27	4.52
PM	13.84	10.21	3.68	10.05	15.99		6.36	3.40	8.34	6.02	4.74	4.86	4.95	7.28	4.23	3.95
WMWR	2.78	7.01	5.35	8.06	17.55	8.84		3.60	8.17	6.62	5.84	3.95	6.46	6.75	5.64	5.91
YNP	11.03	5.10	7.94	8.83	4.78	7.92	8.28		7.35	5.03	4.66	5.00	5.22	6.47	4.00	4.26
EINPW	20.72	11.38	14.80	13.47	9.48	10.76	17.81	10.66		3.53	1.48	1.15	1.21	1.08	1.52	2.06
MBS	9.82	6.00	11.53	8.11	4.99	13.15	8.92	5.11	5.64		1.45	1.31	1.11	1.85	1.70	1.55
WBNP	13.74	7.09	10.86	8.21	7.72	7.70	12.34	7.44	1.45	3.28						
GR	18.48	10.28	15.05	13.37	10.86	9.59	17.00	10.15	0.72	6.09			0	0	0	0
LB	13.23	6.92	11.46	6.15	7.12	8.44	13.29	7.16	2.96	4.11		2.31		0	0.08	0
NL	20.57	9.85	16.77	10.45	6.90	14.88	19.19	11.41	1.49	4.26		2.25	2.01		0.96	0.77
PL	14.43	6.70	10.33	8.48	7.39	7.88	11.97	7.50	1.51	3.07		1.35	1.75	1.90		0.20
SW	10.55	8.01	9.10	9.14	11.14	5.13	9.46	7.36	4.04	4.68		2.82	2.69	5.39	1.29	

also found that the $(\delta\mu)^2$ measure was less reliable than other methods for determining distances using microsatellites.

Discussion

The bison populations exhibited levels of microsatellite variability similar to other mammalian species, especially those recently undergoing population bottlenecks (Roy et al. 1994; Paetkau and Strobeck 1995; Houlden et al. 1996). Bison were also found to have variability levels similar to other large mammalian species in a study of allozymes (McClenaghan et al. 1990). The pre-bottleneck microsatellite variation must surely have been larger than it is today, as bottlenecks lower the amount of genetic variation in a population.

Wood Buffalo National Park was the most variable population when using mean number of alleles, while Custer State Park was the most variable with the other measures. This may be due to the fact that mean number of alleles tends to increase with number of individuals sampled. Wood Buffalo National Park had over two times the number of sampled individuals than the other populations, which may have increased the number of alleles detected. It is also of interest to note that each of the three sampled populations (Pink Mountain and Mackenzie Bison Sanctuary, and the wood bison at Elk Island National Park) which were started from one of the other sampled populations (plains bison at

Elk Island National Park, and Wood Buffalo National Park, respectively) contain less genetic variation than their founding herds. Pink Mountain had a larger number of founders than either of the other two populations, and is closer to its founding population in variability because of this.

The correlations of the mean number of alleles and probability of identity with the number of park founders, and mean number of alleles with the number in the original stock suggest that the amount of variation present in populations is indeed affected by the amount of potentially different genetic material in the populations. The number of founders has more of an effect on the amount of genetic variability in a population than the number of strains in that population does. Therefore, increasing the number of founders for a population is more effective in raising the amount of genetic variability in that population than increasing the number of strains. The failure of mean heterozygosity to correlate with any of the measures of original population size could be due to the fact that a number of the mean heterozygosity values are quite similar and may not be significantly different from one another, resulting in an incorrect ranking.

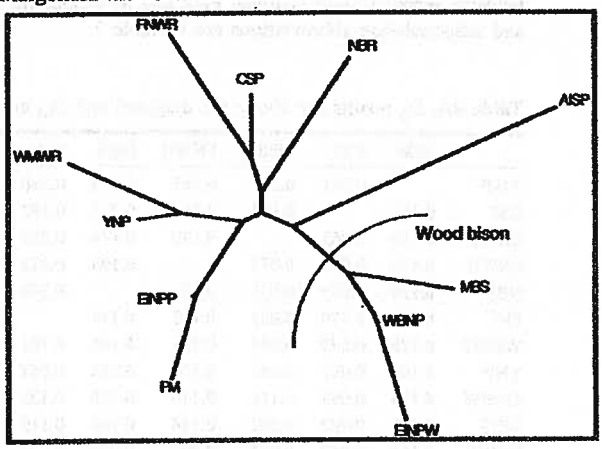
None of the variability measures were found to correlate with the number of mitochondrial alleles found in a study by Polziehn et al. (1996). It must be remembered that the mitochondria is only a single locus inherited maternally, and as such may not be a good indicator of the amount of variation present in populations which have undergone recent size changes.

Some individuals in the Custer State Park population have cow mitochondrial DNA (Polziehn et al. 1995). Known sizes of the loci used in this study in cattle are given in Table 3. Data from Bishop et al. (1994) involve the use of about 200 animals, while Moore et al. (1992) used 19 cattle. Most of the loci, when used in cattle, display a much wider range in allele sizes than bison. None of the bison at Custer State Park contained an allele in their genotype unique to their population, and all the alleles were within the size range found in other bison populations. Therefore, there is no evidence that any microsatellite alleles in the Custer State Park population originated from cattle.

Results of the *G*-test and the assignment test show that all the sampled bison populations are genetically distinct from one another. The founder effect and genetic drift, resulting from the small number of transfers between herds that have occurred, are probably responsible for the uniqueness of these populations. Most of the incorrect assignations of the assignment test were between Pink Mountain and the plains bison at Elk Island National Park, or between Wood Buffalo National Park and Mackenzie Bison Sanctuary or the wood bison population at Elk Island National Park. These are the only three instances of one sampled herd being directly established from another sampled herd, and all three occurred relatively recently.

The largest distances observed in this study were similar to those between widely separated North American polar bear populations, another mammal with a large home range (Paetkau et al. 1995). Founding effects and limited gene flow inflate genetic distance values, so we would expect genetic distances between bison populations to be larger than those obtained. If the bison inhabiting North America before their near-extinction were essentially acting as a single metapopulation, with gene flow occurring between all areas, genetic distances between areas would be low. We would then expect to see low genetic distances between present herds despite the founding effects that have occurred. The extensive natural exchange of animals between herds on a daily basis within public parks today (Lott and Minta 1983; Van Vuren 1983) supports the idea of extensive gene flow in the past. Seton (1910) claimed that all of the plains bison present in Canada acted as one herd, at least before 1869. Roe (1970), also impressed with the homogeneity of bison, stated "in spite of the wide climatic variation, we are confronted with a species which is, broadly speaking, the same throughout this huge territory," with the possible exception of the wood bison. The physical similarity of North American bison should also be reflected in a genetic homogeneity, as is seen here.

Fig. 1. Nei's minimum unrooted tree using the Fitch and Margoliash method. Abbreviations can be found in Table 1.



As the D_S, D_M, and D_{LR} distance measures and both methods of designing trees all resulted in the same unrooted tree, there is some support for the relationships therein. The tree and the D_S, D_M, and D_{LR} distance measures all show the plains bison populations at Elk Island National Park and Pink Mountain to be the most closely related. Since the Pink Mountain population was founded recently from a fairly large number of Elk Island National Park individuals, this is not surprising. This suggests that the founding size of 48 animals for the Pink Mountain population was sufficient to obtain a representative sample of the genetic content of the Elk Island National Park population, though the genetic variation at Pink Mountain is smaller. If mountain bison existed and made a significant contribution to the gene pool of the bison at Yellowstone National Park, we would expect this population to be on a branch by itself or amongst the wood bison populations, as both mountain bison and wood bison were considered *Bison bison athabascae*. The genetic distances between the Yellowstone bison and the other populations would also be expected to be larger. As neither of these are supported by our results, the bison indigenous to Yellowstone were probably not mountain bison, but rather plains bison driven to the area by hunters. The relatively large genetic distances between the Antelope Island State Park pop-

ulation and all other bison populations, and its position on a branch by itself on the unrooted tree, could be a result of the extremely low genetic variability at Antelope Island. Low genetic variation increases the genetic distance between populations, as they may not share the same alleles by chance.

Genetic distances between wood and plains bison populations were larger than those within either of the two proposed subspecies. The three wood bison populations also form one group on the tree, and the genetic distances between these populations are low, relative to other bison populations. This is expected since the Mackenzie Bison Sanctuary and Elk Island National Park wood bison herds were founded solely from Wood Buffalo National Park. This grouping of the wood bison is strong, even after the introduction of numerous plains bison to Wood Buffalo National Park. The wood bison would surely have been even more distinct genetically from the plains bison had the introduction of plains bison to Wood Buffalo National Park not occurred. The clustering of these three populations implies that wood bison are functioning as entities distinct from plains bison, and should continue to be managed separately. The small genetic distances between these populations supports the idea that the founders of Elk Island National Park and Mackenzie Bison Sanctuary were wood–plains hybrids like the animals at Wood Buffalo National Park, and not pure wood bison.

It may be noted that the wood bison population at Elk Island National Park has larger genetic distances using all measures between itself and plains bison populations, while distances between Wood Buffalo National Park and the plains bison populations are smaller. This does not necessarily mean that the wood bison at Elk Island National Park are most like pure wood bison. This population was essentially founded from 11 individuals, as all of the animals shipped to the park from Wood Buffalo National Park were destroyed and only their offspring were kept. The reduction in founding stock could have increased the genetic distance between Elk Island National Park and all other populations. The power of the founder effect to lead to genetically different populations is illustrated by the genetic distance between Mackenzie Bison Sanctuary and the wood bison population at Elk Island National Park. These two populations were started at about the same time with animals taken from the same locale, but their genetic distance shows that their gene pools are quite distinct. Distances between Wood Buffalo National Park and the plains bison populations would be expected to be smaller than those of the other wood bison populations as Wood Buffalo National Park has a much higher genetic variability, and would share more alleles with the plains bison populations by chance.

The *G*-test indicates that the allele frequencies of the Pine Lake subpopulation at Wood Buffalo National Park are significantly different from all other subpopulations, and that the allele distributions at Needle Lake are also different from Sweetgrass and Garden River. All other comparisons were not significantly different. Pine Lake was the area chosen in Van Zyll de Jong et al. (1995) to be the most intermediate between wood bison and plains bison. The Pine Lake region was the site of the initial release of plains bison into the park, and this could have resulted in the uniqueness of this subpopulation, if it contains more genetic input from the introduced plains bison than other regions. However, genetic distances between all the regions of the park are extremely small. This suggests that while there may be differentiation between some of the subpopulations, there is still gene flow between all regions of the park, and no region should be free of genetic input from the introduced plains bison. Of the five animals from Wood Buffalo National Park misassigned with the assignment test to plains bison populations, three were from Sweetgrass, one was from Pine Lake, and one was from Little Buffalo. This also suggests that plains bison genetic material occurs throughout Wood Buffalo National Park. Even though Sweetgrass was chosen by external characteristics to be the most like pure wood bison (Van Zyll de Jong et al. 1995), more bison were assigned to plains bison populations from this region than from any other.

The correlation of the founding size of the bison populations with the mean number of alleles and overall probability of identity shows that microsatellites are good tools for examining the recent history of populations. Populations started from small numbers of animals have less genetic variation. Since the number of strains was not correlated with any of the measures of variation, it is more important to use a large number of animals irrespective of their origin to start herds with high genetic variation. The Pink Mountain population, founded relatively recently from 48 individuals, seems to contain a representative amount of the variation present in its parent population, judging from genetic distance data. This number of founders may then be a minimum level that could be aimed for when new bison populations are started. As the wood bison populations at Elk Island National Park and Mackenzie Bison Sanctuary do not contain as much variation as their founding population, Wood Buffalo National Park, they would not be suitable replacements if the latter population is to be extirpated. All wood bison herds existing today, either inside or outside Wood Buffalo National Park, contain some plains bison genetic material in their gene pool.

Acknowledgements

We thank D. Paetkau for advice concerning data analysis and J. Brzustowski for programming. Thanks also to our anonymous reviewers. Operating grants from Parks Canada and NSERC were provided to C.S., and G.W. has been awarded an NSERC scholarship.

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3). Thus, deletion of JNK2 in macrophages was sufficient to decrease atherogenesis.

Two receptors appear to be essential in foam cell formation and receptor-mediated binding and uptake of modified lipoproteins: CD36 and scavenger receptor A (SR-A) (*20*). Immunofluorescence analyses revealed that expression of CD36 was unchanged in acLDL-stimulated peritoneal $ApoE^{-/-}$ $JNK2^{-/-}$ macrophages (Fig. 4A and fig. S7A). However, analyses with antibodies to SR-A showed increased abundance of this receptor (Fig. 4B and fig. S7B) ($P < 0.01$). Protein immunoblotting confirmed increased abundance of SR-A in protein extracts prepared from macrophages stimulated with acLDL. Amounts of SR-A were not altered in response to acLDL in double knockout or control animals (fig. S7C). $ApoE^{-/-}$ $JNK2^{-/-}$ macrophages formed filopodia-like projections, which were not observed in controls (Fig. 4C). This cellular phenotype is associated with increased adhesion and has been described in macrophages overexpressing SR-A (*21*). To examine whether increased abundance of SR-A in cultured macrophages also occurred in vivo, we used immunohistochemistry to detect SR-A on plaques from $ApoE^{-/-}$ $JNK2^{-/-}$ mice and $ApoE^{-/-}$ control mice. Increased amounts of SR-A were detected in macrophages in plaques of $ApoE^{-/-}$ $JNK2^{-/-}$ mice compared to those of control mice (Fig. 4D).

Alternative splicing results in three types of SR-A transcripts in humans. Occurrence of the Type III SR-A blocks modified LDL uptake (*22*). Therefore, we analyzed the expression of all three splicing variants in macrophages by semiquantitative RT-PCR using specific primers. We could not detect Type III mRNA in macrophages of either genotype. Type I and Type II mRNA was not increased in the absence of JNK2 (fig. S7D). Expression of CD36 or peroxisome proliferator–actived receptor (PPARγ) (*23*), was also not affected. Activation of the well-known JNK target c-jun in aortas from $ApoE^{-/-}$ and $ApoE^{-/-}$ $JNK2^{-/-}$ mice fed either a normal or high-cholesterol diet was not affected, suggesting that c-jun-dependent gene expression was not impaired (fig. S7E). Phosphorylation of SR-A on specific serines is essential for SR-A–dependent processing of modified LDL and for surface expression of SR-A (*24–26*). We immunoprecipitated SR-A from total protein extracts of $JNK2^{-/-}$ macrophages and corresponding wild-type cells. Western blotting of immunoprecipitated SR-A revealed an increased amount of SR-A in $JNK2^{-/-}$ cells compared to wild-type cells (Fig. 4, E and F). Blotting with phosphoserine-specific antibody indicated that the amount of serine-phosphorylated SR-A was lower in $JNK2^{-/-}$ extracts even though more SR-A protein was present (Fig. 4E). We confirmed decreased phosphorylation of SR-A after labeling of $JNK2^{-/-}$ macrophages with [^{32}P] orthophosphoric acid (Fig. 4F).

In this study, we provide in vivo evidence that JNK2 is required in a mouse model of atherogenesis. At the molecular level, we propose that JNK2-dependent decrease of SR-A phosphorylation and increase in SR-A abundance may lead to decreased internalization and degradation of receptor-bound modified LDL and as a consequence to reduced foam cell formation. Indeed, macrophage-specific overexpression of SR-A has been shown to be sufficient to reduce atherosclerosis in ApoE-deficient mice (*27*). In conclusion, specific inhibition of JNK2 activity may provide a therapeutic approach to decrease atheroma formation in patients.

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28. Macrophages were pulse-labeled with [^{32}P]orthophosphoric acid in sodium-phosphate-deficient culture medium 12 hours before harvesting.

29. We thank P. Lerch who provided us with oxLDL; D. Zhang and P. Meier, who helped us with isolation of aortas; H. Joch and A. Jaschko, who helped us with smooth muscle cell isolation; P. Bargsten, who helped us with bone marrow transplantation experiments; W. Krek and P. J. Gough, with whom we had very fruitful scientific discussions; and R. Eferl, for critical reading. Supported by the Swiss National Science Foundation (grant no. 3100-068118), the European Union (grant no. GSRD-CT-2001-00532), the Bundesamt für Bildung und Wissenschaft, and the Swiss Heart Foundation, and the "Forschungskredit 2003" of the University of Zurich.

Supporting Online Material
www.sciencemag.org/cgi/content/full/306/5701/1558/DC1
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23 June 2004; accepted 13 October 2004

Rise and Fall of the Beringian Steppe Bison

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The widespread extinctions of large mammals at the end of the Pleistocene epoch have often been attributed to the depredations of humans; here we present genetic evidence that questions this assumption. We used ancient DNA and Bayesian techniques to reconstruct a detailed genetic history of bison throughout the late Pleistocene and Holocene epochs. Our analyses depict a large diverse population living throughout Beringia until around 37,000 years before the present, when the population's genetic diversity began to decline dramatically. The timing of this decline correlates with environmental changes associated with the onset of the last glacial cycle, whereas archaeological evidence does not support the presence of large populations of humans in Eastern Beringia until more than 15,000 years later.

Climatic and environmental changes during the Pleistocene epoch [from 2 million years ago (Ma) to 10,000 years before the present (ky B.P.)] played an important role in the distribution and diversity of modern plants and animals (*1*, *2*). In Beringia, local climate

and geology created an ice-free refugium stretching from eastern Siberia to Canada's Northwest Territories (*3*). Periodic exposure of the Bering Land Bridge facilitated the exchange of a diverse megafauna (such as bison, mammoth, and musk ox) supported by tundra-steppe grasses and shrubs (*3*, *4*). Humans are believed to have colonized North America via this route, and the first well-accepted evidence of human settlement in Alaska dates to around 12 ky B.P. (*5*). The latest Pleistocene saw the extinction of most Beringian megafauna including mammoths, short-faced bears, and North American lions. The reasons for these extinctions remain unclear but are attributed most often to human impact (*6*, *7*) and climate change associated with the last glacial cycle (*8*).

Pleistocene bison fossils are abundant across Beringia and they provide an ideal marker of environmental change. Bison are believed to have first entered eastern Beringia from Asia during the middle Pleistocene [marine oxygen isotope stages (MISs) 8 to 6, circa (ca.) 300 to 130 ky B.P.] and then moved southward into central North America during the MIS 5 interglacial period (130 to 75 ky B.P.), where they were distributed across the continental United States (*9*). During this time, Beringian and central North American bison populations may have been periodically separated by glacial ice that formed over most of Canada (*10*, *11*). The timing and extent of genetic exchange between these areas remain unclear (*2*).

The abundance and diversity of bison fossils have prompted considerable paleontological and archaeological research into their use as stratigraphic markers. Extensive morphological diversity, however, has complicated discrimination between even the most accepted forms of fossil bison, and the lack of stratigraphy in Beringian sites has prevented the development of a chronological context. These complications create a complex literature of conflicting hypotheses about bison taxonomy and evolution (*9*, *12*). After a severe population bottleneck, which ocurred only 200 years ago (*13*), two subspecies survive in North America: *Bison bison bison*, the plains bison, and *B. b. athabascae*, the wood bison (*9*, *13*).

To investigate the evolution and demographic history of Pleistocene bison, we collected 442 bison fossils from Alaska, Canada, Siberia, China, and the lower 48 United States (*14*). We used ancient DNA techniques to sequence a 685–base pair (bp) fragment of the mitochondrial control region (*14*). Accelerator mass spectrometry radiocarbon dates were obtained for 220 samples, which spanned a period of >60 ky (*14*).

The association of radiocarbon dates with DNA sequences enables the calibration of evolutionary rates within individual species (*15*). Bayesian phylogenetic analyses produced an evolutionary rate estimate for the bison mitochondrial control region of 32% per million years (My) [95% highest posterior density (HPD): 23 to 41% per My] (*14*). This estimate is independent of paleontological calibrations but agrees with fossil-calibrated rates for cattle of 30.1% per My (*16*) and 38% per My (*17*). This rate was used to calculate the ages of key nodes in the bison genealogy (*14*). The most recent common ancestor (MRCA) of all bison included in this analysis lived around 136 ky B.P. (95% HPD: 164 to 111 ky B.P.). In the majority (66%) of estimated trees, Eurasian bison cluster into a single clade, with a MRCA between 141 and 89 ky B.P. Although

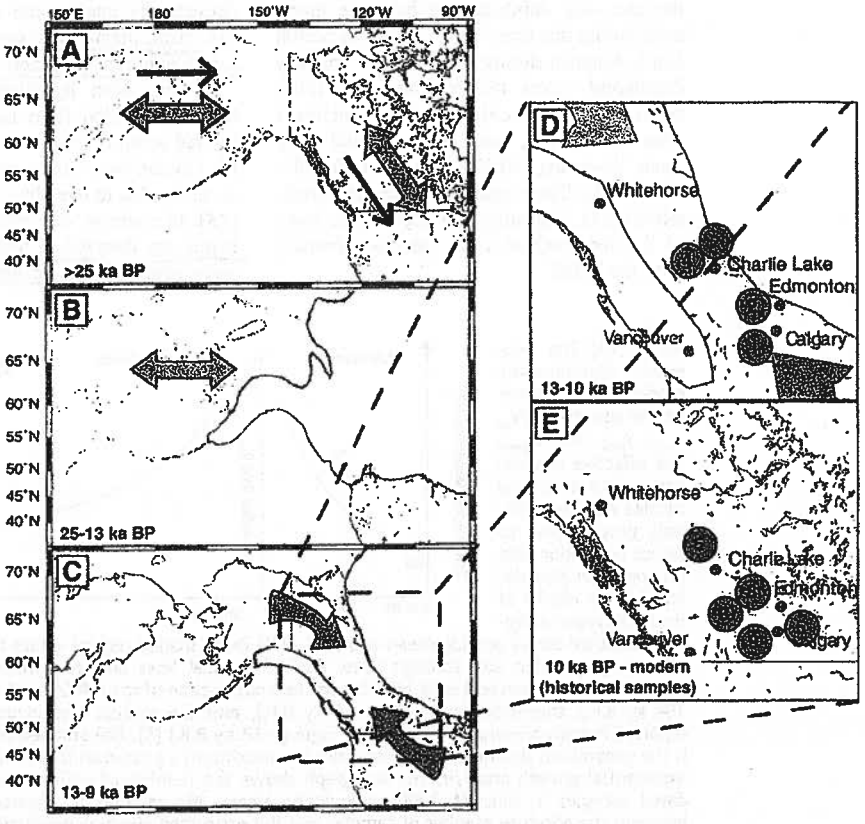

Fig. 1. Distribution of bison in Beringia and central North America through time. (A to C) Double-headed arrows show gene flow between regions. Black arrows indicate colonization events. Circles in maps (D) and (E) designate either northern (red) or southern (blue) ancestry and the number of samples from that location.

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these two estimates overlap, the age of the MRCA of Eurasian bison was the same as that of the root in 4.8% of 135,000 posterior genealogies (with a Bayes factor of 20.83 that the Eurasian MRCA is not also the MRCA of all clades), suggesting that the Eurasian clade is not the oldest in the tree. This suggests that late Pleistocene bison from the Ural Mountains to northern China are descendants of one or more dispersals from North America. Several North American lineages fall within the Eurasian clade, indicating subsequent asymmetric genetic exchange, predominantly from Asia to North America.

Figure 1A depicts inferred gene flow between bison populations in Beringia and central North America during MIS 3 (~60 to 25 ky B.P.), which is the interstadial period before the Last Glacial Maximum (LGM, ca. 22 to 18 ky B.P.). Bison were continuously distributed from eastern Beringia southward into central North America during this period, before the formation of the Laurentide (eastern) and Cordilleran (western) ice sheets created a barrier to north-south faunal exchange. Although any coalescence between these ice masses was brief (*11*), the absence of faunal remains aged 22 to12 ky B.P. (Fig. 1B) (*18*) indicates that the area was uninhabitable by large mammals during this time. Bison fossils in central North America during the LGM are sparsely distributed across the continent (*9*). DNA could be retrieved only from two specimens from this period, both from Natural Trap Cave, Wyoming (20,020 ± 150 and 20,380 ± 90 ky B.P.). These specimens are not closely related (*14*), indicating that populations south of the ice retained some genetic diversity until the LGM.

The ice sheets began to retreat around 14 ky B.P., forming an ice-free corridor (IFC) through which dispersal between Beringia and North America could occur. The first observed bison haplotypes in the IFC are southern in origin (Fig. 1, C and D), with the oldest specimen being in southern Alberta by 11.3 ky B.P., and others near Athabasca, northern Alberta, by 10.4 ky B.P. This finding is consistent with evidence that the first faunal assemblages and archaeological presence in the IFC were southern in origin (*18–20*). The opening of the northern end of the IFC saw a limited southward dispersal of Beringian bison, with a subset of the northern diversity found near the Peace River (northwestern British Columbia) by 11.2 to 10.2 ky B.P. (Fig. 1C) (*14*). Southern bison are also found in this area around 10.5 ky B.P., making it the only location where post-LGM northern and southern clades occurred at the same time. Subsequent genetic exchange between Beringia and central North America was limited by the rapid establishment of spruce forest across Alberta around 10 ky B.P. (*21*) and by the widespread development of peatland across western and northwestern Canada (*22*). North of these ecological barriers, grasslands were reduced by invading trees and shrubs, yet despite the decrease in quality and quantity of habitat (*3*), bison persisted in eastern Beringia until a few hundred years ago (*14*, *23*).

It has been hypothesized that modern bison descended from Beringian bison that moved south through the IFC after the LGM (*9*, *19*) and have since undergone a decline in diversity due to over-hunting and habitat loss (*13*). In contrast, our data show that modern bison are descended from populations that were south of the ice before the LGM and that diversity has been restricted to at least 12 ky B.P., around the time of the megafaunal extinctions. All modern bison belong to a clade distinct from Beringian bison. This clade has a MRCA between 22 and 15 ky B.P., which is coincident with the separation of northern and southern populations by the western Canadian ice barrier. This clade diverged from Beringian bison by 83 to 64 ky B.P. and was presumably part of an early dispersal from Beringia, as indicated by the long branch separating it from Beringian bison (*14*). If other remnants of these early dispersals survived the LGM, they contributed no mitochondrial haplotypes to modern populations.

Coalescent theory is used to evaluate the likelihood of a demographic history, given plausible genealogies (*24*). Under a coalescent model, the timing of divergence dates provides information about effective population sizes through time. To visualize this for bison, a technique called the skyline plot was used (*14*, *25*). The results showed two distinct demographic trends since the MRCA, suggesting that a simple demographic model, such as constant population size or exponential growth, was insufficient to explain the evolutionary history of Beringian bison. We therefore extended the Bayesian coalescent method (*26*) to a two-epoch demographic model with exponential population growth at rate r_{early}, until a transition time, t_{trans}, after which a new exponential rate, r_{late}, applies until the present effective population size, N_0, is reached (Fig. 2A). In this model, both the early and late epochs can have positive or negative growth rates, with both the rates and the time of transition estimated directly from the data.

The analysis strongly supported a boom-bust demographic model (Table 1), in which

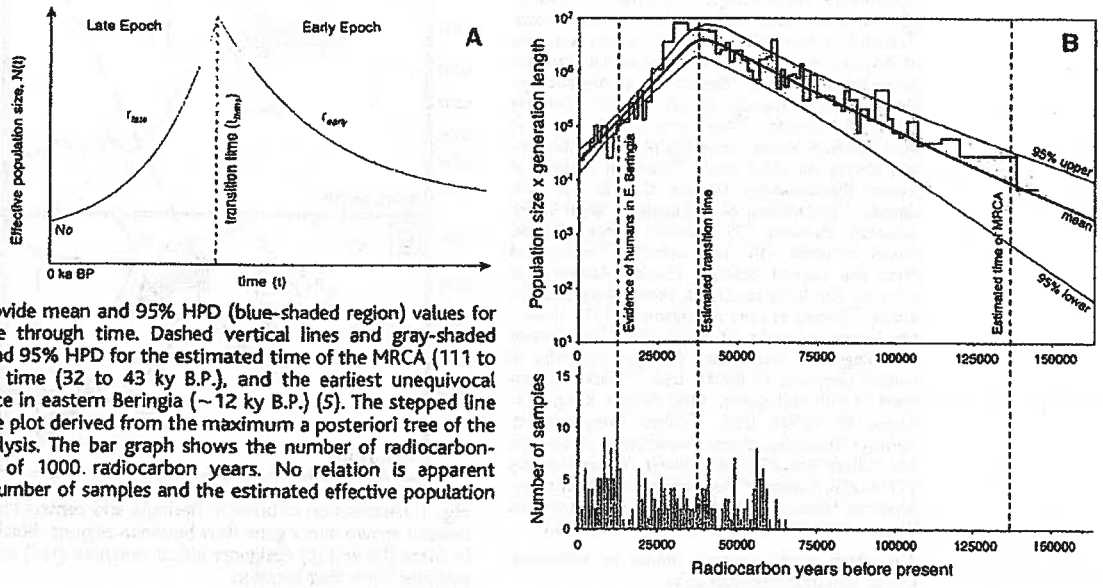

Fig. 2. (A) The two-epoch demographic model with four demographic parameters: N_0, r_{early}, r_{late}, and t_{trans}. The effective population size is a compound variable considered linearly proportional to census population size. (B) Log-linear plot describing the results of the full Bayesian analyses. Smoothed curves provide mean and 95% HPD (blue-shaded region) values for effective population size through time. Dashed vertical lines and gray-shaded regions describe mean and 95% HPD for the estimated time of the MRCA (111 to 164 ky B.P.), transition time (32 to 43 ky B.P.), and the earliest unequivocal reported human presence in eastern Beringia (~12 ky B.P.) (*5*). The stepped line is the generalized skyline plot derived from the maximum a posteriori tree of the exponential growth analysis. The bar graph shows the number of radiocarbon-dated samples in bins of 1000 radiocarbon years. No relation is apparent between the absolute number of samples and the estimated effective population size or transition time.

Table 1. Results of Bayesian analyses assuming constant population size, exponential growth, and a two-epoch model for the full analysis of 191 bison associated with finite radiocarbon dates (*14*). Model parameters are as defined in (*26*). The large difference between the mean goodness-of-fit statistics [ln(posterior)] indicates that under either the Akaike information criterion or Bayesian information criterion tests, the two-epoch model is a significantly better fit to the data than the simpler models.

	Constant size			Exponential growth			Two epoch		
	Lower	Mean	Upper	Lower	Mean	Upper	Lower	Mean	Upper
Age estimates (yr B.P.)									
Root height	117,000	152,000	189,000	113,000	146,000	181,000	111,000	136,000	164,000
Modern/southern clade	20,200	28,000	36,600	18,600	26,400	35,000	15,400	23,200	32,200
Eurasian clade	85,000	116,000	151,000	83,000	112,000	144,000	89,000	114,000	141,000
Model parameters									
Mean ln(posterior)		−6530.795			−6517.35			−6394.568	
Mutation rate (substitutions/site/year)	2.79×10^{-7}	3.78×10^{-7}	4.85×10^{-7}	2.30×10^{-7}	3.20×10^{-7}	4.13×10^{-7}	2.30×10^{-7}	3.20×10^{-7}	4.13×10^{-7}
Kappa	19	27	37	19	27.4	37	19	27	37
Shape parameter	0.22	0.35	0.49	0.22	0.35	0.49	0.22	0.35	0.5
Proportion of invariant sites	0.33	0.45	0.56	0.33	0.45	0.56	0.34	0.45	0.56

an exponential expansion of the bison population was followed by a rapid decline, with a transition around 37 ky B.P. (Fig. 2B). At the height of the boom, the population size was around 230 times (95% HPD: 71 to 454 times) that of the modern population. When this model is applied to the modern clade alone, a growth period peaks around 1000 years ago (95% HPD: 63 to 2300 yr B.P.) and is followed by a rapid decline (*14*), which is consistent with historical records of a population bottleneck in the late 1800s (*13*). These results illustrate the power of this method to recover past demographic signals.

The effects of population subdivision and patch extinction and recolonization on coalescence patterns have not been fully characterized, yet they can influence demographic estimates such as skyline plots (*27*). To test for the effect of population subdivision on our models, the two-epoch analysis was repeated first without the Eurasian bison and then without both Eurasian and central North American bison. The results of these analyses were consistent with those for the entire data set (*14*), suggesting that the assumption of panmixia does not affect the analysis. These results suggest that the major signal for the boom-bust scenario came from the well-represented eastern Beringian population.

The timing of the decline in Beringian bison populations (Fig. 2B) predates the climatic events of the LGM and events at the Pleistocene-Holocene boundary. The bison population was growing rapidly throughout MIS 4 and 3 (~75 to 25 ky B.P.), approximately doubling every 10,200 (95% HPD: 7500 to 15,500) years. The reversal of this doubling trend at 42 to 32 ky B.P. and the subsequent dramatic decrease in population size are coincident with the warmest part of MIS 3, which is marked by a reduction in steppe-tundra due to treecover reaching its late Pleistocene maximum (*28*). Modern boreal forests serve as a barrier to bison dispersal because they are difficult to traverse and provide few food sources (*3*). After the interstadial, cold and arid conditions increasingly dominated, and some component of these ecological changes may have been sufficient to stress bison populations across Beringia. Previous reports of local extinction of brown bears (*29*) and hemionid horses (*8*) in Alaska around 32 to 35 ky B.P. support the possibility of a larger scale environmental change affecting populations of large mammals.

These results have considerable implications for understanding the end-Pleistocene mass extinctions, because they offer the first evidence of the initial decline of a population, rather than simply the resulting extinction event. These events predate archaeological evidence of significant human presence in eastern Beringia (*5*), arguing that environmental changes leading up to the LGM were the major cause of the observed changes in genetic diversity. If other species were similarly affected, differences in how these species responded to environmental stress may help to explain the staggered nature of the megafaunal extinctions (*7*, *30*). However, it is possible that human populations were present in eastern Beringia by 30 ky B.P., with reports of human-modified artifacts as old as 42 to 25 ky B.P. from the Old Crow basin in Canada's Yukon Territory (*31*). Although the archaeological significance of these specimens is disputed and the number of individuals would be low, the specimens are consistent with the timing of the population crash in bison. This emphasizes that future studies of the end–Pleistocene mass extinctions in North America should include events before the LGM.

Ancient DNA is a powerful tool for studying evolutionary processes such as the response of organisms to environmental change. It should be possible to construct a detailed paleoecological history for late Pleistocene Beringia using similar methods for other taxa. Almost none of the genetic diversity present in Pleistocene bison survived into Holocene populations, erasing signals of the complex population dynamics that took place as recently as 10,000 years ago.

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MANAGEMENT STRATEGIES FOR CONSERVATION OF GENETIC DIVERSITY IN WOOD BISON *(Bison bison athabascae)*

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2006

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File Report No. 135

Executive Summary

Conservation of genetic diversity is essential to the long-term survival of any species, particularly in light of changing environmental conditions. Reduced genetic diversity may negatively impact the adaptive potential for a species. In addition, low genetic diversity leads to an increased risk of inbreeding effects, through the uncovering of deleterious recessive alleles. Consequently, management of genetic diversity is an important component of recovery strategies for threatened and endangered wildlife.

In Canada, the single greatest limiting factor affecting recovery of the threatened wood bison (*Bison bison athabascae)* is the presence of bovine tuberculosis *(Mycobacterium bovis)* and brucellosis *(Brucella abortus)* in and around Wood Buffalo National Park (WBNP). Despite the successful salvage of the founders for the Elk Island National Park (EINPW) and Mackenzie bison populations in the 1960s, and most recently the Hook Lake Wood Bison Recovery Project (HLWBRP), the majority of wood bison genetic diversity exists within the diseased populations of the Greater Wood Buffalo National Park Ecoregion. Genetic diversity in the Mackenzie and EINPW wood bison populations is substantially less than the wild populations from which they were salvaged, likely due to a combination of the founder effect and genetic drift. In addition, disease-free wood bison herds that have been established through national recovery efforts have been generally managed as small and genetically isolated populations, although some herds have received supplemental releases from the wood bison herd at EINPW. Thus, due to a series of founding events and population bottlenecks, genetic diversity is not well distributed among disease-free wood bison herds in Canada.

In this study, we used a simulation modeling approach to evaluate strategies for management of genetic diversity and maintenance of gene flow among disease-free wood bison herds. Within a metapopulation framework, we evaluated the relative effects of population size, number of populations, movement of animals between populations, and harvesting or culling regimens on genetic diversity. Based on current population genetic status and the influence of these factors on genetic diversity in simulated populations, we arrived at the following conclusions:

- Additional genetic salvage should be conducted from diseased bison in and around Wood Buffalo National Park to ensure that genetic diversity of wood bison is well represented and conserved in disease-free populations. Each salvage effort should be based on a large number of founding individuals, similar to the effort undertaken at the HLWBRP.
- The most genetically important disease-free populations should be the primary source for creating new disease-free populations.
- The HLWBRP represented one of the most genetically important populations because it was unrelated to EINPW and because it was established with a larger number of founders. However, genetic management of this population must continue to ensure diversity is not quickly lost due to its small size.[1]
- Herd size is the primary factor affecting the loss of diversity from the wood bison metapopulation through time. Management of individual wood bison herds

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[1] Unfortunately, a single case of bovine tuberculosis was confirmed in the HLWBRP in June 2005 (see Lutze-Wallace *et al.*, 2006). Subsequent disease testing and culling up until winter 2006 had confirmed that an additional five bison were infected with bovine tuberculosis. In March 2006, all remaining HLWBRP bison were destroyed.

above a minimum population size (i.e., census size $\geq$ 400 individuals) will minimize the loss of diversity.

- The movement of animals among all herds will significantly reduce the rate at which diversity is lost. However, assurance that populations remain large should take precedence over gene flow when populations are below carrying capacity.

Given the long-history of the northern diseased bison issue and the potential for infectious diseases to undermine conservation objectives, it will be equally important for wildlife managers to consider and balance genetic management objectives of wood bison, with disease and health management objectives. This will require objective and more quantitative assessments of risks and opportunities when considering translocation of bison for genetic management purposes.

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1.0 Introduction

The recovery of wood bison (*Bison bison athabascae*) in northern Canada will ultimately depend on conservation and management of genetic diversity among free-ranging populations of bison that are free from infection with bovine tuberculosis *(Mycobacterium bovis)* or brucellosis *(Brucella abortus)* (see Gates *et al.* 2001). In order for real progress in wood bison recovery to be made, wildlife managers will need to develop and implement recovery strategies that are centered on both disease eradication and genetic conservation objectives (see Connelly *et al.* 1990, Nishi *et al.* 2002b, Wilson *et al.*, 2005, Shury *et al.* 2006).

Here, our broad intent is to define the issues and develop strategies that are relevant to genetic conservation of wood bison. One of our primary motivators for conducting this work was to respond to the general need for a genetic management strategy as identified by the National Wood Bison Recovery Team (Gates *et al.* 2001), and the Governments of Yukon (Government of Yukon 1998) and British Columbia (Harper *et al.* 2000), which have formally recognized the importance of genetic management and augmentation within their respective wood bison recovery plans.

Another important motivator for this work was the Hook Lake Wood Bison Recovery Project (HLWBRP) (see Gates *et al.* 1998, Nishi *et al.* 2001, Nishi *et al.* 2002a, Wilson *et al.*, 2005). At the time we initiated this project, we were anticipating that captive-born bison from the HLWBRP might become available in the near future for national wood bison recovery efforts (see Nishi *et al.* 2002a and 2002b, APFRAN 2003, Nishi *et al.* 2004). As the HLWBRP wood bison represented a new source of genetic diversity that was salvaged from the Slave River Lowlands (Wilson 2001, Wilson *et al.*

2005), the recovery project represented a potentially valuable source for augmenting genetic diversity of existing conservation herds. However, prior to this project there was no well-developed rationale or genetic management strategy upon which specific recommendations for genetic augmentation of other wood bison conservation herds could be based.

Unfortunately, in June 2005, bovine tuberculosis was confirmed in a 3 year-old captive-born bull at the HLWBRP (Lutze-Wallace *et al.*, 2006). Subsequent disease testing and a preliminary epidemiological investigation in which 21 animals were euthanized indicated that at least 6 animals were infected with bovine tuberculosis (B. Elkin and J. Nishi, unpublished data). All remaining founder and captive-born animals were culled on site, or transported to abattoirs in Alberta in February and March 2006. However, for this report, we have included the HLWBRP according to our original research project design and analyses, because the results and implications are still relevant; indeed, the loss of the HLWBRP emphasizes the importance of genetic salvage for wood bison.

We suggest that there are two basic issues that are important for the genetic conservation of the threatened[2] wood bison in Canada. The first issue involves the management of genetic diversity within (Wilson and Zittlau 2004, Wilson *et al.*, 2005, Wilson *et al.*, in prep) and among disease-free[3] wood bison populations. The second is the relatively low proportion of genetic variability that is represented by existing disease-

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[2] Wood bison (*Bison bison athabascae*) are considered a threatened subspecies of North American Bison by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC); they are listed in Appendix II by the Convention on the International Trade In Endangered species (CITES).

[3] We use the term "disease-free" and "healthy" to describe bison herds that are free from infection with either bovine tuberculosis *(Mycobacterium bovis)* or brucellosis *(Brucella abortus).*

free wood bison populations, compared to the diseased metapopulation in Wood Buffalo National Park (WBNP) (see Wilson and Strobeck 1999).

Disease-free wood bison herds established through national recovery efforts have been generally managed as relatively small (see Wilson and Zittlau 2004) and genetically isolated populations. Although some herds have received supplemental releases from the wood bison national recovery herd at Elk Island National Park (EINPW) (Gates *et al.,* 2001), it is likely that the founder effect and/or genetic drift has affected the levels of genetic diversity in some or all of these herds. Apart from the Mackenzie population, all other disease-free wood bison herds in Canada (Aishihik, Etthithun, Nordquist, Nahanni, Hay-Zama, Chitek Lake, Caribou Mountains-Lower Peace area, Waterhen and Syncrude) originate either directly or indirectly from the EINPW population. As such, we expect those populations to be less genetically variable than EINPW animals and hence, be among the least variable bison populations (Wilson and Strobeck 1999). With the exception of the Mackenzie and EINPW populations, there is a dearth of population genetic data from disease-free wood bison populations. Although those other populations are integral to national recovery efforts, the amount by which their genetic diversity has been reduced is unknown. Thus, it is important to evaluate whether current management of reintroduced, disease-free wood bison, which are maintained as genetically isolated populations, may have a negative impact on genetic diversity and population viability over the long term (see Halbert *et al.*, 2004, 2005). Correspondingly, there is a need to evaluate and develop strategies that would outline whether and how gene flow may be established among disease-free herds.

Despite salvage of healthy wood bison from WBNP in the 1960s to create the Mackenzie and EINPW populations (see Blyth 1995, Gates *et al.* 2001, Nishi *et al.* 2002b), the majority of genetic diversity still exists in the diseased wood bison populations of the Greater WBNP Ecoregion (Wilson and Strobeck 1999, Wilson *et al.* 2005). The genetic diversity in the Mackenzie and EINPW wood bison populations is substantially less than the wild populations from which they were salvaged, largely due to a combination of founder effect and genetic drift (Wilson and Strobeck 1999). Therefore, it is important to determine whether and how much additional genetic diversity from the diseased bison populations should be salvaged to ensure long-term survival and evolution of the subspecies in populations free from infection with bovine tuberculosis and brucellosis. Indeed, resolution of the larger northern diseased bison issue through depopulation of diseased bison and repopulation with healthy bison will likely be strongly linked to a decision on whether sufficient genetic salvage has been or will be achieved (see Shury *et al.* 2006)

In this report we take a broad genetic management perspective on conservation of wood bison in northern Canada, and outline strategies for:

1) establishing and maintaining gene flow between disease-free, reintroduced wood bison populations which historically existed as a panmictic population; and
2) conducting additional genetic salvage of wood bison from the diseased WBNP metapopulation.

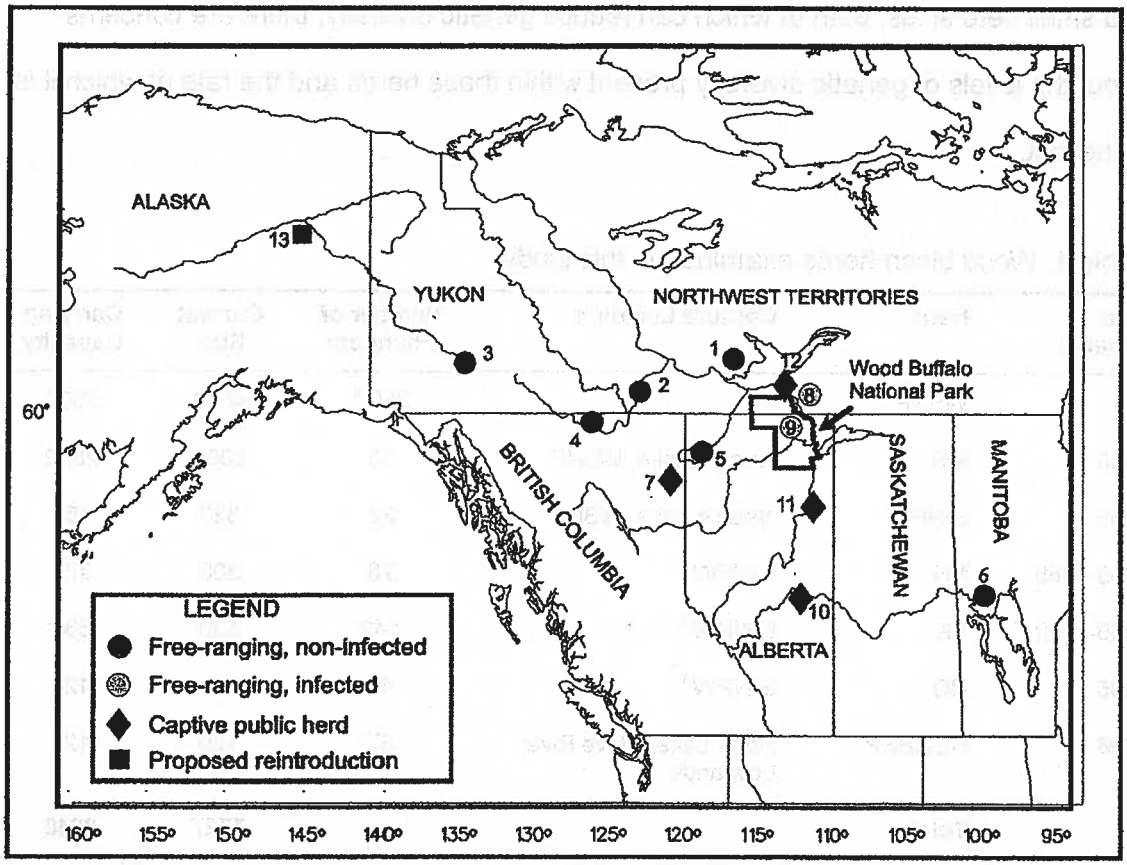
1.1 Background – wood bison

Wood bison declined from an estimated 100,000 to about 250 individuals by the beginning of the 20th century (Soper 1941 and see reviews by Gates *et al.* 1992, Stephenson *et al.* 2001, and Reynolds *et al.* 2003). By 1900, the geographic range of wood bison had shrunk accordingly, and their range was restricted to a part of the area currently known as Wood Buffalo National Park (WBNP). Following the enactment of a federal law in 1893 to protect the remaining wood bison and actual enforcement of the law by the Northwest Mounted Police starting in 1897, the number of bison increased over subsequent years (Soper 1941). By the time WBNP was created in 1922 to protect the remaining wood bison and their habitat (Figure 1) (Lothian 1976, 1979), the population was estimated at between 1500 and 2000 (Siebert and Soper, in Gates *et al.* 1992).

From 1925-1928, over 6,600 plains bison from Buffalo National Park in Wainwright, AB, were transported by rail and barge to WBNP (see Gates *et al.*, 1992, Fuller 2002, McEwan 1995). In addition to the resultant hybridization between the introduced plains bison and the indigenous wood bison, this translocation also introduced bovine tuberculosis (*Mycobacterium bovis*) and brucellosis (*Brucella abortus*) into the WBNP population (van Zyll de Jong 1986, Connelly *et al.*, 1990, Carbyn *et al.* 1993, Gates *et al.*, 2001, Fuller 2002, Joly and Messier 2004a).

The combined effect of disease (tuberculosis and brucellosis) and predation by wolves is hypothesized to be an important regulating factor in the population dynamics of WBNP bison (Messier 1989, Gates 1993, Gates *et al.* 1997, Joly and Messier 2004b, Joly and Messier 2005, but also see Carbyn *et al.*, 1993, Carbyn *et al.* 1998, Bradley

and Wilmshurst 2005). From a wildlife management perspective, the presence of diseased bison is considered to be the single greatest factor limiting the recovery of wood bison in northern Canada (Gates *et al.* 2001).



1 Mackenzie (MB)
2 Nahanni (NH)
3 Yukon (YK)
4 Nordquist (NQ)
5 Hay Zama (HZ)
6 Chitek Lake (CL)
7 Etthithun Lake (EL)
8 Slave River Lowlands (SRL)
9 Wood Buffalo National Park (WBNP)
10 Elk Island National Park (EINP)
11 Syncrude Canada Ltd. (SN)
12 Hook Lake Wood Bison Recovery Project (HLWBRP)
13 Yukon Flats (proposed)

Figure 1. Wood bison herds in Canada.

There have been three attempts to salvage disease-free wood bison from the WBNP area (see Blyth 1995, Gates *et al.*, 2001, and Nishi *et al.*, 2002b, Wilson *et al.*, 2005). In the 1960s herds were established from WBNP wood bison at the Mackenzie Bison Sanctuary (MB) and at EINPW. Areas adjacent to WBNP were the source for the HLWBRP (Figure 1, Table 1). However, due to an unrepresentative number of founders and small herd sizes, both of which can reduce genetic diversity, there are concerns about the levels of genetic diversity present within these herds and the rate at which it is being lost.

Table 1. Wood bison herds examined in this study.

Year Initiated	Herd	Capture Location	Number of Founders	Current Size	Carrying Capacity
-	WBNP	-	250 [a]	4500	5000
1963	MB	Needle Lake, WBNP	16	2000	2000
1965	EINPW	Needle Lake, WBNP	22	320	450
1980-1989	NH	EINPW	70	200	320
1986-1992	YK	EINPW [b]	142	530	530
1995	NQ	EINPW [b]	49	62	120
1996	HLWBRP	Hook Lake, Slave River Lowlands	57	120	125
	Total	-	-	**7727**	**8040**

[a] In this case, "founders" refers to the lowest number of animals estimated for this population in approximately 1900 (Soper 1941).

[b] Animals were also received from Moose Jaw Wild Animal Park and/or the Toronto Metro Park, both of which were derived from EINPW.

1.2 Background – genetic diversity

Conservation of genetic diversity is an essential aspect of the management of threatened and endangered species. Genetic diversity is vital for population viability. In

the short term, low levels of diversity can result in inbreeding depression, increasing the probability of population extirpation or reducing population fitness (Coulson *et al.* 1998, Saccheri *et al.* 1998, Puurtinen *et al.* 2004). The effects of inbreeding can accumulate over many generations, as the frequency of slightly deleterious alleles can gradually increase over time due to genetic drift (Lande 1994, Lande 1995, Lynch *et al.* 1995, Whitlock 2000). This is a particular concern in small populations, where natural selection can be inefficient for alleles that have only slight effects on fitness (Wright 1977).

Over the long-term, a paucity of genetic diversity will reduce the population's ability to adapt to changing environmental conditions and respond to natural selection pressures (Franklin 1980, Lacy 1987, Frankham *et al.* 1999). Furthermore, once unique genetic material is lost from a species it cannot be regained, even through the process of mutation. Genetic diversity can be lost through founder effects, population bottlenecks, genetic drift, and selection. The rate at which genetic diversity is lost will depend on the population's size and degree of isolation; small, isolated populations can lose genetic diversity within a few generations, whereas large, continuous populations may not lose significant amounts of diversity over thousands of years (e.g., Zittlau 2004). In small populations where genetic drift is most rapid, the fixation of common alleles will result in the reduction of genetic diversity.

If gene flow is inhibited, the diversity that is lost within a population each generation will not be replenished from other populations. Current management strategies treat wood bison herds as isolated units, with no gene flow occurring among them, suggesting they will be vulnerable to this phenomenon. The small size of many

wood bison populations makes them highly susceptible to the processes of genetic drift. However, as drift in subdivided populations can result in the fixation of different alleles in each population, overall metapopulation diversity can be higher in this scenario, as fixation of an allele guarantees that it will not be lost (Kimura and Crow 1963). Consequently, the proper management strategy to maximize diversity at the metapopulation level is not clear without further knowledge of the populations' demographic and genetic composition.

1.3 Measuring genetic diversity

Some commonly used measures of diversity are expected heterozygosity, probability of identity, allelic richness, the number of private alleles, and allelic proportion. Expected heterozygosity measures the proportion of a population that is expected to possess different alleles at a particular locus (Nei and Roychoudhury 1974). As such, it is a measure of the amount of variance in allele frequencies, and is maximized when these frequencies are equivalent across a locus. Unbiased probability of identity measures the probability that two individuals within a population have the same genotype (Paetkau *et al.* 1998). Probability of identity is also a measure of the evenness with which alleles are distributed at a locus. Allelic richness measures the mean number of alleles at a locus, weighted against the sample size of the population (El Mousadik and Petit 1996). Private alleles are alleles that occur only in a single population and, therefore, give a measure of the distinctiveness of each population (Kalinowski 2004). Finally, allelic proportion measures the proportion of alleles within a metapopulation that are found in each subpopulation. Populations that contain a large

amount of the diversity found within a metapopulation have a high allelic proportion value.

As a variety of genetic diversity measures exist, identification of the best measure is frequently debated. Many conservation geneticists feel that allelic richness is the most relevant measure of genetic diversity, because a large number of alleles will supply a source of variation upon which selection can act (e.g., Schoen and Brown 1993, Bataillon *et al.* 1996, Petit *et al.* 1998). Generally, selection of the most useful genetic diversity measures will be largely dependent on the problem being addressed. For example, information on expected heterozygosity, probability of identity, and allelic richness can be useful for determining which herds may be in danger of suffering inbreeding effects. Identification of private alleles and allelic proportion can be especially useful for identifying potential sources of highly variable individuals to add to any genetically depauperate herds.

1.4 Current population genetic status of wood bison herds

Lack of knowledge concerning population genetic diversity can complicate the implementation of genetic management efforts. Although previous studies have shown that the three populations of wood bison captured from the WBNP ecoregion are less variable than their founding population (Wilson and Strobeck 1999, Wilson *et al.* 2005), the diversity within any conservation herd founded from EINPW is currently unknown.

Attempts were made to collect samples from wood bison populations founded from EINPW for this study. However, where this was not possible, attempts were made to elucidate current levels of diversity through simulation techniques. Programs such as GENELOSS (England and Osler 2001) employ Monte Carlo sampling methods to simulate

the amount of diversity lost when populations are subjected to a bottleneck. As founding events essentially act as bottlenecks, this program is useful for estimating levels of diversity in populations for which DNA samples are unavailable. GENELOSS has proven valuable in estimating the magnitude of population bottlenecks in elk populations, based on current levels of diversity (Williams *et al.* 2004).

Determining the genetic importance of populations to metapopulation diversity is valuable for revealing where management efforts and resources can be best applied. If WBNP is to be depopulated and replaced with healthy wood bison, as recommended by a Federal Environmental Review Panel (Connelly *et al.* 1990, and see Shury *et al.* 2006), the genetic importance of this population to the wood bison subspecies must be examined to determine whether additional salvage attempts are required to sufficiently sample the genetic diversity in this region. Furthermore, potential sources of individuals from which to establish new conservation herds can be recognized if genetic importance is well understood. Due to differences in levels of diversity, all populations are not equally capable of responding to changes in environmental conditions. Therefore, an evaluation of the contribution of each population to the total metapopulation diversity, while simultaneously accounting for genetic divergence from other populations, was used to identify populations that have the highest evolutionary-response potential (Petit *et al.* 1998).

1.5 Estimating future diversity of wood bison

Due to forces such as genetic drift, differential reproductive success, and natural selection, an eventual decline in genetic diversity is unavoidable in natural populations when the process of mutation is not considered. Mutation occurs at an average annual

rate of 2.2×10^{-9} per base pair in mammalian genomes (Kumar and Subramanian 2002), and is only occasionally a significant force. In contrast, drift, differential reproduction, and selective forces can have substantial impacts on a population's ability to retain diversity over time. The effect of different management strategies on the rate at which genetic diversity is lost can be modeled using population viability analyses (PVA). This capacity, and its ability to estimate the probability of population extinction, has resulted in PVA becoming a critical tool for wildlife conservation and management.

PVA software packages can project the future viability of natural populations based on the effects of deterministic and stochastic processes. A number of PVA packages exist, which vary with respect to their goals, data requirements, and assumptions (for review, see Wilson *et al.* 2003). Previously, the PVA program VORTEX (Lacy *et al.* 2003) was determined to be the most suitable for estimating the future diversity of wood bison (Wilson *et al.* 2003). The VORTEX model closely resembles the life history of bison and can accommodate the large amount of information available for wood bison populations. The more information incorporated into a model, the greater the predictive ability it will have. VORTEX has been used to estimate the probability of population extinction in a bison population (Halbert *et al.* 2004), as well to evaluate strategies for minimizing loss of diversity in two Elk Island National Park bison populations (Wilson and Zittlau 2004).

VORTEX can model dispersal and translocation of animals among herds, allowing for the evaluation of management strategies at a metapopulation level. The definition of metapopulation used here follows Thomas and Gray (2002), who describe it as a group of populations among which actual or potential movements of animals can occur. As

most wood bison populations have been established and managed as isolated units, anthropogenic barriers, and not adaptive or genetic differences, primarily limit dispersal among populations. Consequently, the wood bison metapopulation is defined as all public herds of wood bison. While few reports exist of wood bison movements throughout their range prior to their decline in the 1800s, it is likely that all regions were joined by at least occasional animal movements (Roe 1970, Reynolds *et al.* 2003). Therefore, the designation of a single wood bison metapopulation in Canada is warranted.

Proper conservation of the threatened wood bison requires an evaluation of the effectiveness of various strategies for genetic management. We used VORTEX to outline potential management strategies for maximizing the retention of genetic diversity over the next 500 years. We also used VORTEX to estimate the genetic effects of establishing gene flow among wood bison herds.

1.6 Objectives

By integrating analyses of genetic diversity and population viability, we:

1. determined the levels of genetic diversity in all populations, either from previous studies, the collection and analysis of DNA samples, or the use of simulation studies.
2. calculated the genetic importance of wood bison populations to determine how diversity is distributed throughout the metapopulation, and established the importance of the diseased WBNP animals to the total metapopulation diversity.

3. modeled the rate of change, over the next 500 years, in levels of genetic diversity for the wood bison populations in WBNP, EINPW, MB, HLWBRP, Yukon (YK), Nahanni (NH), and Nordquist (NQ).
4. modeled the rate of change, over the next 500 years, in levels of genetic diversity for the entire wood bison metapopulation.
5. evaluated various management options with respect to their effect on the genetic diversity of the metapopulation and on the projected degree of relatedness among subpopulations. These management scenarios are described further in Section 2.4, *Management scenarios modeled.*

2 Methods

2.1 Genotype analyses

To examine the genetic diversity in various salvaged herds of wood bison, tissue samples from the Yukon population were obtained and compared with previously published data from the EINPW, MB, WBNP, and HLWBRP populations (Wilson and Strobeck 1999). Where possible, the EINPW dataset was expanded from 36 to 218 individuals, isolated from a previous study on parentage, which included approximately 95% of the adults in the 1998 population (Wilson *et al.* 2002).

DNA was isolated from YK wood bison tissue samples using a QIAamp® Tissue Extraction Kit (QIAGEN Inc., Mississauga, ON). The DNA was analyzed at the same 11 microsatellite loci used in previous bison studies (Wilson and Strobeck 1999, Wilson *et al.* 2005) to ensure that the results were directly comparable. The expanded EINPW dataset was available for five loci: BM2830, BMC1222, and BM1225 (Bishop *et al.* 1994), BOVFSH (Moore *et al.* 1992), and RT29 (Wilson and Strobeck 1999). This expanded dataset was used in all population-level analyses described below, but was not used for the individual-level analyses due to the large number of unknown genotypes that would result. Primers were fluorescently labeled with FAM, HEX, or TET dye groups, and PCR conditions were as in Wilson and Strobeck (1999). PCR products were visualized on an ABI 377 DNA Sequencer.

2.2 Genetic diversity

Loci examined in the YK population were tested for heterozygote deficiencies using a Markov chain algorithm in GENEPOP 3.4 (Raymond and Rouset 1995). Linkage

disequilibrium between loci was also examined using GENEPOP 3.4. As BM4513 was monomorphic, this locus was not included in the pairwise tests for linkage disequilibrium. Error rates for both tests were adjusted to 0.05 using a Dunn-Sidák correction (Sokal and Rohlf 1995). Genetic variation was measured as allelic richness, average unbiased expected heterozygosity (Nei and Roychoudhury 1974), overall unbiased probability of identity (Paetkau *et al.* 1998), number of private alleles, and allelic proportion. These values were compared with those derived for the previously examined wood bison populations of WBNP, EINPW, and MB.

A G-test and an assignment test were used to determine the distinctiveness of the YK population from other wood bison populations. The G-test was performed using pairwise comparisons between YK and all other wood bison populations, summed over all loci (Sokal and Rohlf 1995). The assignment test (Paetkau *et al.* 1995; available at http://www.biology.ualberta.ca/jbrzusto/Doh.php) was performed among all genotyped wood and plains bison populations as described in Wilson *et al.* (2005).

Genetic distance measures can reveal the level of relatedness between populations. Nei's standard genetic distance, D_S (Nei 1973), was calculated among all wood bison populations using a program available at http://www.biology.ualberta.ca/jbrzusto/GeneDist.php. A neighbour-joining unrooted tree (Saitou and Nei 1987) created with PHYLIP 3.61 (Felsenstein 1995) was used to visualize genetic distances.

For any population where DNA samples were unavailable, Monte Carlo sampling was performed using the program GENELOSS (England and Osler 2001) to estimate the effects of the bottleneck or founding event on genetic diversity. First, however,

GENELOSS was used to estimate diversity in the YK population. These results were then compared to results of DNA analyses, in order to test the ability of GENELOSS to accurately estimate genetic diversity in bison populations.

GENELOSS requires information on initial allele frequencies at all loci of interest within a population, the number of effective breeding pairs during the bottleneck, and the generational length of the bottleneck. As the YK herd was primarily founded from the wood bison at EINPW, allele frequencies from the latter population could be used to approximate initial values prior to the bottleneck. The number of effective breeding pairs in the YK population was estimated knowing that the effective size of bison populations is approximately 1/3 that of the actual size (Wilson and Zittlau 2004). From 1986 to 1992, 142 wood bison from EINPW were obtained for the YK herd (Gates *et al.* 2001, Table 1). The effective number of founders for this population is, therefore, approximately 47 and the number of effective breeding pairs, rounded to the nearest whole number, is 24. As the YK population received an influx of founders over a six-year period, estimating the number of generations since its inception is not straightforward. However, if we assume a generation time of five years based on the minimum age of male reproduction observed in wood bison at EINPW (Wilson *et al.* 2002), between two and four generations have passed since the establishment of the YK population. Consequently, simulations were performed with bottleneck durations of between two and five generations. Simulated values for allelic diversity and heterozygosity were considered significantly different from those observed in YK if the latter values were not included within the 95% confidence interval. Three thousand iterations were performed for each scenario.

The NH and NQ populations were also founded from EINPW wood bison (Table 1), so allele frequencies from EINPW could be used as initial allele frequencies for these population simulations as well. The NQ population was founded from 49 individuals in 1995, but only 36 individuals were counted in 1996 (Gates *et al.* 2001). Therefore, either six or eight breeding pairs founded this population, and two or three generations have passed since its inception. The history of the NH population is substantially more complicated than that for either the YK or NQ populations. After being founded in 1980, individuals were added to NH in 1989 (from Moose Jaw Wild Animal Park) and 1997 (from EINPW; Gates *et al.* 2001). The NH population has also undergone numerous fluctuations in size. Consequently, estimating the number of founding breeding pairs requires several assumptions for which little information is available. As such, any estimates of diversity for NH based on our current knowledge of its number of founders are likely unreliable and, therefore, this population was not included in the GENELOSS analyses.

The contribution of each population to the genetic diversity of the metapopulation was measured using the program CONTRIB (Petit *et al.* 1998). While traditional measures of genetic diversity, such as those mentioned above, can assess the levels of genetic variation within a number of populations, they do not reveal the relative conservation value of the diversity present within populations. Two measures of genetic diversity can be directly compared across populations: unbiased expected heterozygosity, and allelic richness. As the number of alleles sampled from a population increases with sample size, variance in sample size must be allowed for if allelic richness is to be used for determining genetic importance. This was done using

rarefaction, a technique for sampling alleles in each population to allow for sample size differences (Hurlbert 1971). Rarefaction was performed with 25 individuals, as this value must be lower than the smallest population sample size (Table 3). The contribution to overall diversity was also partitioned into two components: the proportion resulting from the diversity of the population of interest, and the proportion due to the divergence of the population from all others (Petit *et al.* 1998). As locus BM4513 was monomorphic in the wood bison populations examined, it was not included in this analysis. The genetic importance of each population was also calculated after the removal of WBNP from the data set to determine the relative importance of the disease-free wood bison populations to metapopulation genetic diversity.

2.3 Simulating loss of diversity over time

VORTEX 9.40 (Lacy *et al.* 2003) was used to model the change in genetic diversity over time for individual herds and the wood bison metapopulation. Locations of the seven herds examined are indicated in Figure 1. VORTEX simulates the change in genetic diversity under the effects of various deterministic and stochastic processes. Although VORTEX has high data requirements, there is considerable information regarding bison available from historical records, annual census data, and published literature. The availability of demographic data varies greatly among the herds, and input variables were determined according to data quality and abundance (Table 2). For populations with little or low-quality data, input variables were estimated from the literature or from the EINPW herd, for which a vast amount of detailed demographic data is available (Table 2). The carrying capacity for each herd that was not heavily

managed was estimated from the range size and habitat productivity (Table 2). All estimates were based on predictions made in 2003.

2.4 Management scenarios modeled

We projected the change in genetic diversity resulting from four scenarios:

1. Present demographic distributions were used to project the future diversity according to current harvesting regimens and carrying capacities. Scenarios were modeled from the date of herd establishment until the year 2500, with founding animals taken from the source populations indicated in Table 1.
2. Individual herds were modeled independently to project the loss of genetic diversity over time when all alleles in the founding individuals are considered to be unique. This would demonstrate the extent to which genetic diversity has been lost from these herds due to founding effects during their establishment. These results can be contrasted to Scenario 1, in which each population's alleles are sampled from their source population of either ElNPW or WBNP.
3. The value of maintaining multiple herds within the metapopulation was examined by modeling the existence of different numbers and combinations of herds. Results were then compared to the existing scenario with all seven herds belonging to the metapopulation. This projection also indicates each herd's contribution to metapopulation diversity. The modeled combinations are as follows:

    a) The metapopulation was modeled according to demographic distributions if only WBNP had been established.

b) The metapopulation was modeled according to demographic distributions if only WBNP, EINPW and MB had been established.

c) The metapopulation was modeled according to demographic distributions if all but the HLWBRP herd had been established.

d) The removal of WBNP was modeled to project the impact that a depopulation of the WBNP herd would have on the genetic diversity of the metapopulation. The entire age- and sex-classes were harvested in the year 2020, 120 years since the herd's establishment.

4. Metapopulation management options were evaluated by modeling the influence of various harvesting and translocation strategies on the change in genetic diversity over the next 500 years.

a) Gene flow among all herds was modeled by annually moving an equal number of animals from each herd, with the exception of WBNP, into each of the other herds. No animals were translocated from WBNP due to the risk of spreading disease. Also, no animals were moved into HLWBRP, because the carrying capacity of HLWBRP was small and the health status of the HLWBRP was still uncertain[4]. Simulated movements of animals ranged from five female and five male calves/year, to ten female and ten male calves/year. Only calves were used in translocations, and all calves were assumed to survive the translocation process.

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[4] We completed this project under the assertion that the HLWBRP was disease-free and conducted these analyses prior to the confirmation of tuberculosis in the HLWBRP in June 2005 (see Introduction).

b) Movements of animals from HLWBRP were modeled to project the effect of increased gene flow from this herd on the overall diversity of the metapopulation. A total of 20 male and female calves from HLWBRP were moved annually into the MB, EINPW, NH, YK, and NQ herds. Only calves were used in translocations, and all calves were assumed to survive the translocation process.

c) As MB has the largest population size of the disease-free herds and is thus most able to contribute animals to other populations without suffering size reductions, the genetic effects of moving animals from this herd were also examined. A total of 20 male and female calves from MB were moved annually into the EINPW, NH, YK, and NQ herds. Only calves were used in translocations, and all calves were assumed to survive the translocation process.

d) To examine the genetic contribution of the NH, YK, and NQ herds, gene flow among only these herds was examined. In addition, gene flow from all herds, except WBNP, into NH, YK, and NQ was modeled, as well as movements of bison from only EINPW, MB, and HLWBRP into only NH, YK, and NQ. A total of 20 male and female calves were moved annually, only calves were used in translocations, and all calves were assumed to survive the translocation process.

e) The genetic implications of additional salvage attempts were modeled by establishing one to three new herds of wood bison, consisting of the same demographic structure as the original HLWBRP herd, either in 2016 (20

years after HLWBRP establishment), or at 10-year intervals in 2016, 2026, and 2036. No gene flow occurred in these scenarios.

Table 2. Source of data for population viability analyses (PVA) of Canadian wood bison herds.

	Source of Data for Each Herd						
Data	**WBNP**	**MB**	**EINPW**	**NH**	**YK**	**NQ**	**HLWBRP**
Inbreeding rate	Ralls *et al.* 1988	Ralls *et al.* 1988	Ralls *et al.* 1988	Ralls *et al.* 1988	Ralls *et al.* 1988	Ralls *et al.* 1988	Ralls *et al.* 1988
Calf mortality rate	Reynolds *et al.* 2003	Gates and Larter 1990	Herd-specific data	Reynolds *et al.* 2003	Reynolds *et al.* 2003	Reynolds *et al.* 2003	Herd-specific data
Adult & juvenile mortality rate	Fuller 1966	Gates and Larter 1990	Herd-specific data	Fuller 1966	Fuller 1966	Fuller 1966	Herd-specific data
Female reproduction	Fuller 1966	Gates and Larter 1990	Herd-specific data	Fuller 1966, Gates and Larter 1990	Fuller 1966, Gates and Larter 1990	Fuller 1966, Gates and Larter 1990	Herd-specific data
Male reproduction	EINPW [a]	EINPW [a]	Herd-specific data	EINPW [a]	EINPW [a]	EINPW [a]	EINPW [a]
Sex & age distribution	EINPW [a]	Herd-specific data	Herd-specific data	Herd-specific data	Herd-specific data	Herd-specific data	Herd-specific data

[a] Estimated from EINPW data, provided by W. Olson, pers. com. 2003.

3.0 Results

3.1 Existing genetic diversity

3.1.1 Measured genetic diversity

Four of the loci (Eth121, RT24, BOVFSH, and RT29) were found to have a deficiency of heterozygotes in the YK population ($P > 0.05$). Only Eth121 was deficient of heterozygotes when the Dunn- Sidák experiment-wise error rate was used ($P > 0.05$). Of the 45 pairwise tests for linkage disequilibrium, four were found to be significant ($P > 0.05$). However, none of these were significant when the Dunn- Sidák experiment-wise error rate was used.

The genetic diversity present in YK and other wood bison populations is shown in Table 3. The WBNP population was the most variable according to all measures. MB had the lowest heterozygosity, while YK was the least variable based on probability of identity and allelic richness measures (without correction for sample size). YK had lower levels of diversity than EINPW, its founding population, based on all measures. There were a total of eight private alleles, seven of which occurred in the WBNP population. A total of 74 alleles were observed in all populations. Of these, a high of 72 (allelic proportion = 0.973) were observed in WBNP and a low of 40 (allelic proportion = 0.541) were observed in YK. When WBNP was excluded from the analysis, the total number of alleles observed decreased to 66, or 0.892 of the total when WBNP is included. The number of private alleles observed in HLWBRP increased to 13 when WBNP was excluded from the analysis, and the allelic proportion increased to 0.924, the highest of all populations sampled (Table 4).

Table 3. Existing genetic diversity in wood bison herds.

Herd	Sample Size	He	1/pI	Allelic Richness	# Private Alleles	Allelic Proportion
WBNP	81	0.552[b]	5.7×10^{7}[b]	6.55[b]	7	0.973
MB	28	0.441[b]	7.6×10^{5}[b]	4.27[b]	0	0.635
EINPW	36/218[a]	0.517	1.4×10^{6}	4.09	0	0.608
YK	26	0.483	7.1×10^{5}	3.64	0	0.541
HLWBRP	57	0.508[b]	5.8×10^{6}[b]	5.55[b]	1	0.824
Total		**0.515**[c]				

[a] Combined estimate from 36 individuals used in Wilson and Strobeck (1999) and, where possible, all adult individuals in the 1998 population.

[b] From Wilson and Strobeck 1999.

[c] Calculated as mean heterozygosity for all populations where this value is known, weighted by total population sizes.

Table 4. Private alleles and allelic proportion when WBNP is excluded from the analysis.

Herd	Private Alleles	Allelic Proportion
MB	3	0.712
EINPW	0	0.682
YK	0	0.606
HLWBRP	13	0.924

3.1.2 Genetic distinctness of populations

Pairwise G-test comparisons revealed that YK was significantly different from all other populations ($P < 0.001$). Despite changes in allelic richness from those reported by Wilson and Strobeck (1999), the extended EINPW dataset was not significantly different from the previous EINPW dataset at the $P > 0.1$ level (Table 3).

Assignment test results are presented in Table 5. Most wood bison (61%) were assigned to the correct population and only four animals were incorrectly assigned across subspecies. All four of these bison were from WBNP. While 73% of the YK

individuals were correctly assigned, a fairly large proportion of them (19%) were misassigned to EINPW.

Table 5. Assignment test results. Numbers in parentheses are the proportion of assigned individuals. Individuals assigned to their own population are in bold. The "plains bison" column includes individuals assigned to any plains bison population.

Source Populations	Sink Populations					
	WBNP	MB	EINPW	YK	HLWBRP	Plains bison
WBNP	**39 (0.48)**	6 (0.07)	4 (0.05)	3 (0.04)	25 (0.31)	4 (0.05)
MB	3 (0.11)	**21 (0.75)**	0	0	4 (0.14)	0
EINPW	2 (0.06)	2 (0.06)	**28 (0.78)**	3 (0.08)	1 (0.03)	0
YK	0	1 (0.04)	5 (0.19)	**19 (0.73)**	1 (0.04)	0
HLWBRP	16 (0.28)	6 (0.11)	0	3 (0.05)	**32 (0.56)**	0

Genetic distances between all wood bison population pairs are in Table 6. Distances were generally smallest between WBNP and the other populations, and largest between MB and the other populations. Distances between HLWBRP and the other populations also tended to be small. The smallest observed distance was between WBNP and the HLWBRP (0.018), and largest was between YK and MB (0.166). The distance between YK and EINPW, at 0.041, was the second smallest observed.

The unrooted neighbour-joining tree depicting the genetic distances among all populations is illustrated in Figure 2. The YK-EINPW nodes are proximately located on this tree, as are the HLWBRP and WBNP nodes. The MB node is farthest from the other external nodes.

Table 6. D_S distances between wood bison populations.

Herd	WBNP	MB	EINPW	YK	HLWBRP
WBNP	0	0.074	0.051	0.063	0.018
MB		0	0.141	0.166	0.058
EINPW			0	0.041	0.074
HLWBRP				0.068	0

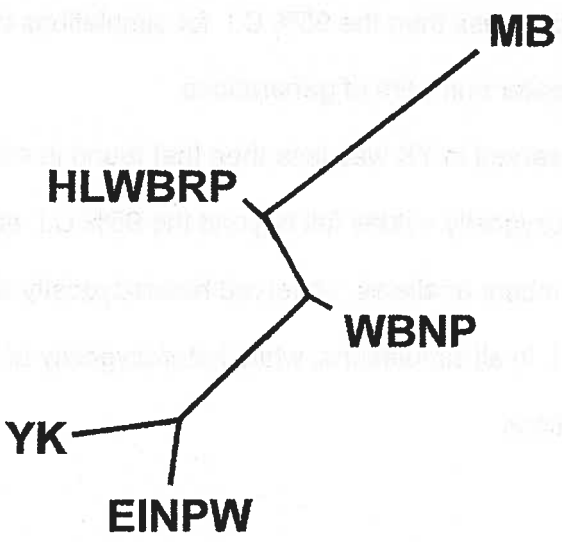

Figure 2. Neighbour-joining unrooted D_S tree of wood bison populations.

3.1.3 Estimated genetic diversity

Numbers of alleles and expected heterozygosity that were observed in the YK population and estimated from GENELOSS simulations for the pre-founding event and five subsequent generations are shown in Tables 7 and 8. Note that the GENELOSS

heterozygosity measure is not unbiased, as were the heterozygosity measures discussed above, and is thus not directly comparable to those values. Locus BM4513 was not included in the simulations, as it only contains a single allele in these populations. Overall, the number of alleles observed in YK was greater than that predicted under any simulated scenario. However, the observed number of alleles fell outside of the 95% C.I. at only two loci. The simulations for locus BM143 consistently had fewer alleles than observed in the YK population. In fact, more alleles were observed in YK at this locus than in EINPW, its founding population. The observed number of alleles at YK locus RT24 was less than the 95% C.I. for simulations of two and three generations, but not for greater numbers of generations.

The overall heterozygosity observed in YK was less than that found in each of the simulations. However, the heterozygosity values fell beyond the 95% C.I. at only two loci. Similar to the results for numbers of alleles, observed heterozygosity at locus BM143 was greater than the 95% C.I. in all simulations, while heterozygosity at RT24 was below the 95% C.I. in all simulations.

Table 7. Numbers of alleles sampled in the YK wood bison population and estimated from GENELOSS simulations for the pre-founding event and five subsequent generations. The number of breeding pairs was set at 24 for all simulations. Values in parentheses are standard deviations. Values in bold are not contained within the 95% C.I., with the observed value being either too high (superscript 'H') or too low (superscript 'L').

Locus	Initial (pre-founding event)	Sampled	Simulated Mean for Number of Generations			
			2	3	4	5
BM2830	6	5	4.31 (0.55)	4.19 (0.54)	4.07 (0.55)	3.96 (0.57)
BMC1222	3	3	3.00 (0.03)	3.00 (0.05)	2.99 (0.10)	2.98 (0.14)
BM1225	5	5	4.88 (0.34)	4.73 (0.47)	4.59 (0.57)	4.45 (0.63)
BOVFSH	9	6	6.83 (0.72)	6.52 (0.81)	6.21 (0.86)	5.97 (0.91)
RT29	7	6	6.03 (0.68)	5.81 (0.69)	5.63 (0.72)	5.50 (0.75)
BM143	3	4	**2.56^{H} (0.50)**	**2.47^{H} (0.50)**	**2.39^{H} (0.49)**	**2.35^{H} (0.48)**
Eth121	3	3	3.00 (0.04)	2.99 (0.09)	2.98 (0.12)	2.97 (0.16)
RT24	3	2	**2.98^{L} (0.13)**	**2.97^{L} (0.18)**	2.93 (0.25)	2.89 (0.31)
RT27	2	2	2.00 (0)	2.00 (0)	2.00 (0)	2.00 (0)
RT9	3	3	2.97 (0.17)	2.93 (0.26)	2.87 (0.33)	2.82 (0.38)
Total	4.4 (2.27)	3.9 (1.52)	3.86 (1.60)	3.76 (1.50)	3.67 (1.41)	3.59 (1.34)

Table 8. Heterozygosity sampled in the YK wood bison population and estimated from GENELOSS simulations for the pre-founding event and five subsequent generations. The number of breeding pairs was set at 24 for all simulations. Values in parentheses are standard deviations. Values in bold are not contained within the 95% C.I., with the observed value being either too high (supercript 'H') or too low (superscript 'L').

Locus	Initial (pre-founding event)	Sampled	Simulated Mean for Number of Generations			
			2	3	4	5
BM2830	0.593	0.556	0.580 (0.052)	0.576 (0.064)	0.570 (0.074)	0.562 (0.083)
BMC1222	0.587	0.551	0.576 (0.038)	0.572 (0.047)	0.565 (0.054)	0.558 (0.063)
BM1225	0.524	0.417	0.512 (0.075)	0.505 (0.088)	0.499 (0.104)	0.495 (0.111)
BOVFSH	0.682	0.697	0.668 (0.057)	0.662 (0.069)	0.655 (0.081)	0.646 (0.090)
RT29	0.752	0.754	0.736 (0.035)	0.727 (0.043)	0.721 (0.051)	0.715 (0.055)
BM143	0.513	0.654	**0.502^{H} (0.021)**	**0.497^{H} (0.030)**	**0.492^{H} (0.036)**	**0.487^{H} (0.041)**
Eth121	0.589	0.532	0.578 (0.034)	0.570 (0.045)	0.565 (0.051)	0.559 (0.056)
RT24	0.511	0.234	**0.501^{L} (0.053)**	**0.496^{L} (0.064)**	**0.491^{L} (0.073)**	**0.486^{L} (0.079)**
RT27	0.444	0.426	0.434 (0.046)	0.530 (0.057)	0.425 (0.065)	0.422 (0.072)
RT9	0.452	0.391	0.444 (0.065)	0.440 (0.076)	0.432 (0.090)	0.428 (0.099)
Total	0.565 (0.097)	0.521 (0.158)	0.553 (0.095)	0.547 (0.094)	0.542 (0.094)	0.537 (0.093)

As the ability of GENELOSS to estimate diversity in bison populations was confirmed by the similarities between observed and simulated values for YK, simulations were also performed to predict the diversity present in the NQ population. Simulated numbers of alleles for this population are shown in Table 9. There is little difference in the number of alleles between the scenario simulated for 2 generations with 6 breeding pairs and the scenario simulated for 3 generations with 8 breeding pairs. All simulated values are lower than those for the YK population.

Simulated levels of heterozygosity in NQ are shown in Table 10. The differences in diversity between the scenario with 6 breeding pairs over 2 generations and 8 breeding pairs over 3 generations are amplified when heterozygosity is examined. As with the number of alleles, all simulated heterozygosity values for NQ are lower than those simulated for the YK population.

Table 9. Pre-founding event number of alleles and simulated number of alleles for the NQ wood bison population. Values in parentheses are standard deviations.

Locus	Initial	Number of Generations			
	(pre-founding event)	2	2	3	3
		Number of Reproductive Pairs			
		8	6	8	6
BM2830	6	3.69 (0.65)	3.44 (0.71)	3.44 (0.70)	3.20 (0.73)
BMC1222	3	2.93 (0.25)	2.86 (0.35)	2.86 (0.35)	2.77 (0.42)
BM1225	5	4.04 (0.76)	3.75 (0.84)	3.71 (0.83)	3.35 (0.88)
BOVFSH	9	5.36 (0.96)	4.87 (1.00)	4.87 (1.00)	4.34 (1.00)
RT29	7	5.12 (0.74)	4.80 (0.79)	4.80 (0.81)	4.47 (0.83)
BM143	3	2.26 (0.44)	2.20 (0.40)	2.19 (0.39)	2.14 (0.35)
Eth121	3	2.91 (0.29)	2.84 (0.36)	2.83 (0.38)	2.74 (0.44)
RT24	3	2.78 (0.41)	2.66 (0.48)	2.67 (0.47)	2.56 (0.51)
RT27	2	2.00 (0)	2.00 (0.03)	2.00 (0.03)	1.99 (0.10)
RT9	3	2.70 (0.46)	2.56 (0.51)	2.58 (0.50)	2.47 (0.53)
Total	4.4 (2.27)	3.38 (1.15)	3.20 (1.01)	3.20 (1.00)	3.00 (0.85)

Table 10. Pre-founding event expected heterozygosity and simulated heterozygosity for the NQ wood bison population. Values in parentheses are standard deviations.

Locus	Initial	Number of Generations			
	(pre-founding event)	2	2	3	3
		Number of Reproductive Pairs			
		8	6	8	6
BM2830	0.593	0.557 (0.090)	0.542 (0.107)	0.539 (0.111)	0.522 (0.124)
BMC1222	0.587	0.553 (0.071)	0.540 (0.083)	0.536 (0.087)	0.523 (0.101)
BM1225	0.524	0.490 (0.124)	0.484 (0.141)	0.473 (0.145)	0.455 (0.164)
BOVFSH	0.682	0.641 (0.097)	0.628 (0.111)	0.622 (0.117)	0.606 (0.125)
RT29	0.752	0.703 (0.063)	0.692 (0.072)	0.683 (0.079)	0.666 (0.090)
BM143	0.513	0.483 (0.049)	0.472 (0.061)	0.467 (0.066)	0.450 (0.082)
Eth121	0.589	0.554 (0.062)	0.541 (0.078)	0.536 (0.080)	0.519 (0.098)
RT24	0.511	0.481 (0.094)	0.469 (0.105)	0.464 (0.109)	0.452 (0.124)
RT27	0.444	0.417 (0.081)	0.407 (0.092)	0.405 (0.097)	0.390 (0.115)
RT9	0.452	0.426 (0.110)	0.414 (0.121)	0.412 (0.127)	0.405 (0.140)
Total	0.565 (0.097)	0.530 (0.091)	0.519 (0.090)	0.515 (0.088)	0.499 (0.087)

3.1.4 Estimated genetic contribution

The contribution of each wood bison population to overall diversity, based on unbiased expected heterozygosity, is presented in Table 11 and Figure 3. Values of heterozygosity shown in Table 11 differ from those reported in Table 3 due to the exclusion of the monomorphic locus BM4513 in the analysis of genetic importance. If the mean heterozygosity is taken from 11 loci (when heterozygosities of monomorphic loci are 0), values become identical to those in Table 3. Populations with a below-average contribution to metapopulation diversity have negative genetic importance

values. WBNP has the highest genetic importance and the largest contribution to the total diversity of wood bison. This is even more evident when only the contribution due to the diversity within each population is considered. WBNP's contribution to genetic diversity is below average when considering population divergence. HLWBRP, MB and YK have negative total genetic importance values, but when the contribution to divergence is not considered, only MB and YK make negative contributions to diversity.

Table 11. Genetic contribution of each wood bison population to the total diversity, measured as unbiased expected heterozygosity, He. C_T represents the total contribution of each population to wood bison diversity, C_S represents the amount of this contribution due to each population's diversity, and C_D represents the amount of this contribution due to each population's divergence.

Herd	He	C_T	C_S	C_D
WBNP	0.586	0.010300	0.024200	-0.013800
MB	0.485	-0.005590	-0.027600	0.022090
EINPW	0.569	0.008300	0.008070	0.000168
YK	0.532	-0.003980	-0.007920	0.004000
HLWBRP	0.559	-0.009130	0.003720	-0.012400

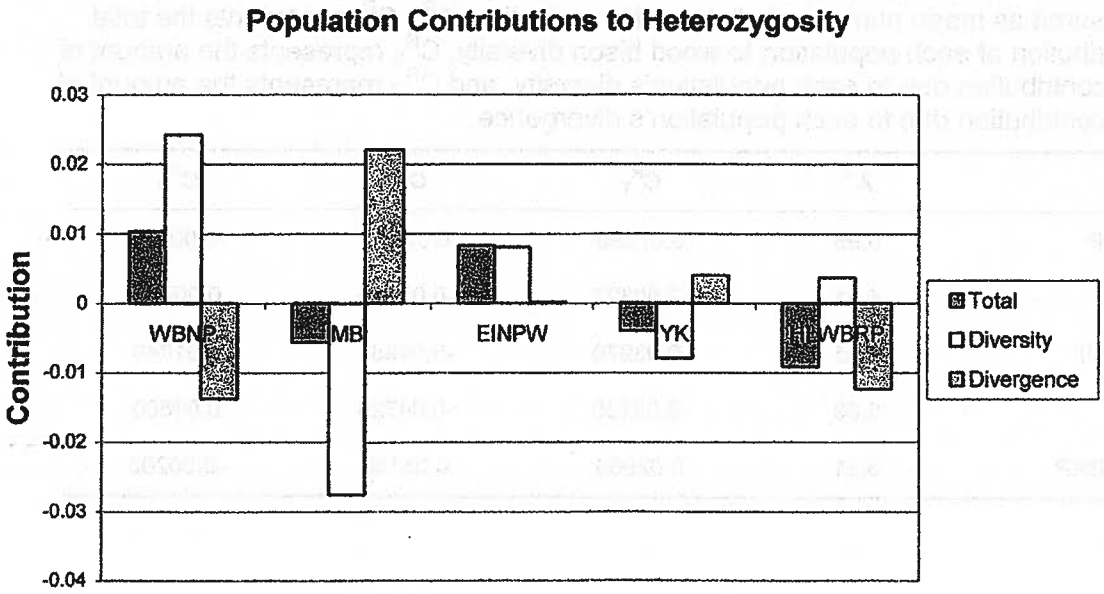

Figure 3. Contributions of each population to total wood bison diversity, measured as unbiased expected heterozygosity.

Allelic richness with rarefaction and each population's contribution to total diversity based on this measure are shown in Table 12 and Figure 4. Calculating allelic richness with rarefaction only slightly changes the order of population ranks compared to ranks determined without weighting for sample size (Tables 3, 12). The WBNP population is the most diverse population based on allelic richness with rarefaction and its contribution to total diversity, followed by the HLWBRP population. This trend is even greater if just the contribution to diversity for each population is considered. However, when divergence is considered, these populations both have a slightly below average contributions to overall diversity. With rarefaction, the EINPW population is the least diverse (compared to being second least diverse based on allelic richness). EINPW, MB, and YK have negative contributions to the total allelic richness diversity, and all contributions are due solely to within-population diversity.

Table 12. Genetic contribution of each wood bison population to total diversity, measured as mean number of alleles with rarefaction, A^R. C^R_T represents the total contribution of each population to wood bison diversity, C^R_S represents the amount of this contribution due to each population's diversity, and C^R_D represents the amount of this contribution due to each population's divergence.

Herd	A^R	C^R_T	C^R_S	C^R_D
WBNP	5.95	0.07020	0.07590	-0.00597
MB	4.51	-0.00327	-0.01050	0.00703
EINPW	3.85	-0.03970	-0.04980	0.01040
YK	3.89	-0.03130	-0.04720	0.01600
HLWBRP	5.21	0.02960	0.03150	-0.00206

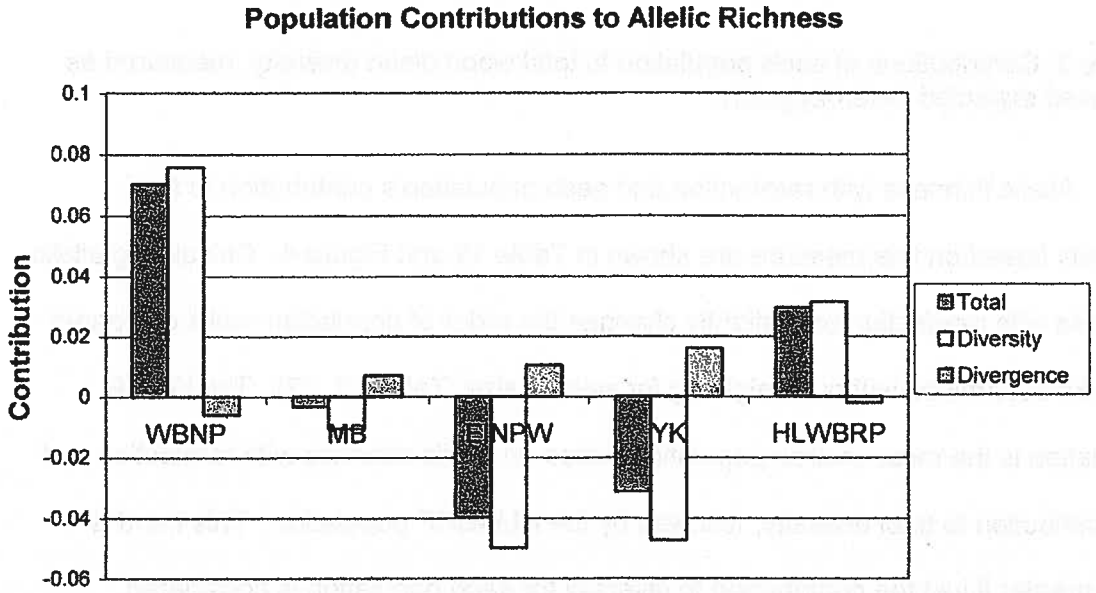

Figure 4. Contributions of each population to total wood bison diversity, measured as allelic richness.

When WBNP is not included in the analysis, only EINPW and, very slightly, MB make positive contributions to the total diversity of wood bison, as measured by expected heterozygosity (Table 13, Figure 5). However, when the contribution from

within-population diversity is separated from that due to divergence, only EINPW and HLWBRP make positive contributions to diversity.

Table 13. Genetic contribution of each wood bison population to the total diversity, measured as unbiased expected heterozygosity when WBNP is excluded from the analysis. Abbreviations are defined in Table 11.

Herd	C_T	C_S	C_D
MB	0.000199	-0.029300	0.029300
EINPW	0.013300	0.019000	-0.005640
YK	-0.004040	-0.002610	-0.001380
HLWBRP	-0.009430	0.012900	-0.022400

Unlike the trend observed for expected heterozygosity, HLWBRP is the only population that makes a positive contribution to diversity based on allelic richness (Table 14, Figure 6). When population divergence is excluded from the calculation, MB also makes a slightly above average contribution to overall wood bison diversity.

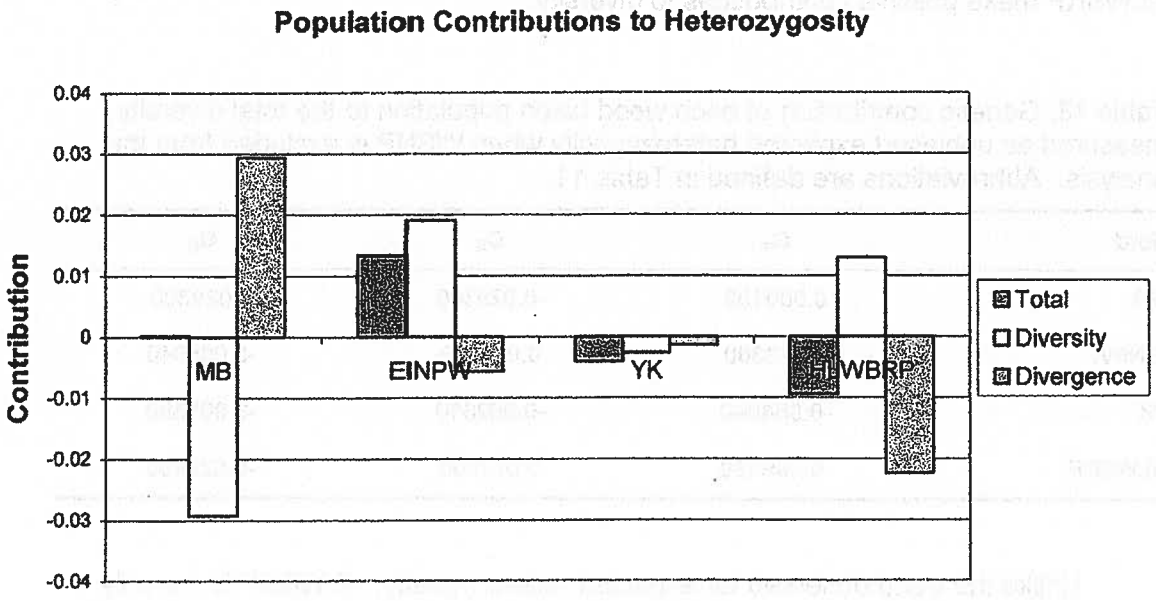

Figure 5. Contributions of each population to total wood bison diversity, measured as unbiased expected heterozygosity, when WBNP is excluded from the analysis.

Table 14. Genetic contribution of each population to overall diversity, measured as mean number of alleles with rarefaction when WBNP is excluded from the analysis. Abbreviations are defined in Table 12.

Herd	C^R_T	C^R_S	C^R_D
MB	-0.003640	0.012900	-0.016600
EINPW	-0.097900	-0.046300	-0.051700
YK	-0.073300	-0.042000	-0.030900
HLWBRP	0.082500	0.075800	0.006570

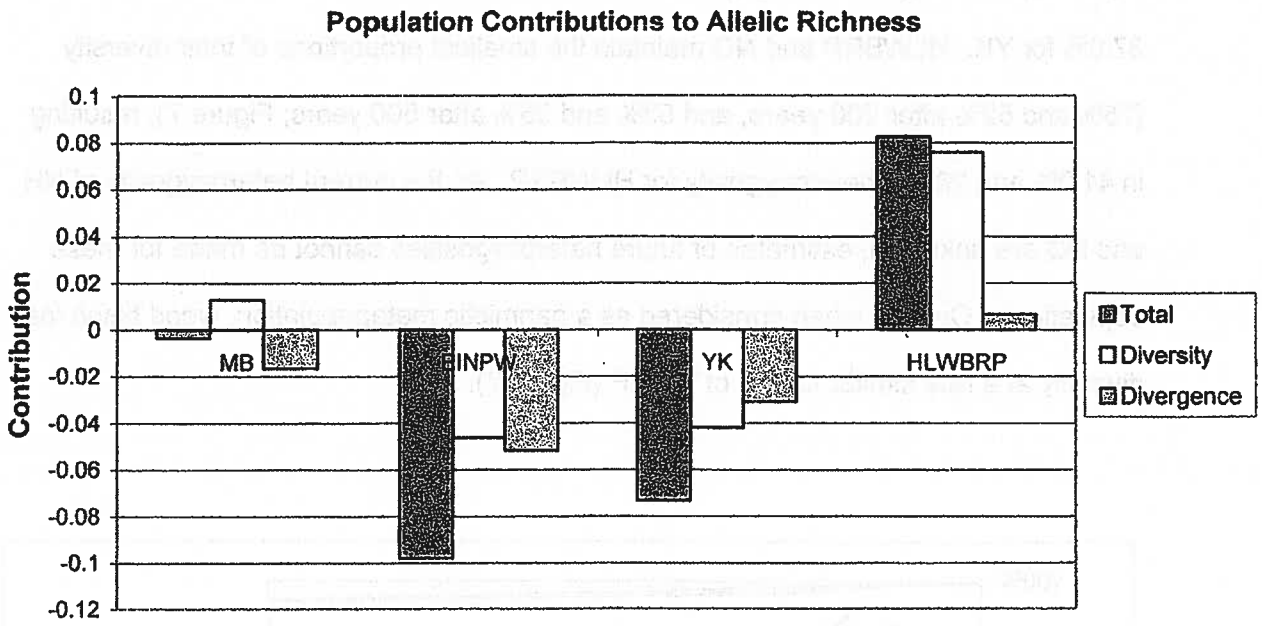

Figure 6. Contributions of each population to total wood bison diversity, measured as allelic richness, when WBNP is excluded from the analysis.

3.2 Future genetic diversity

3.2.1 PVA Scenario 1: Effects of present conditions

When present demographic distributions of each herd were used in the PVA model, along with current harvesting regimens and carrying capacities, WBNP and MB maintain the highest proportion of their existing diversity (Table 15, Figure 7). After 200 years, heterozygosity in WBNP is 99% of its original value and, after 500 years, heterozygosity declined to only 98%. Based on existing diversity, this decline would result in a heterozygosity of 54.4% and 53.8% after 200 and 500 years. Heterozygosity in the MB herd declined to 89% of its original value after 200 years (to 39.4%), and to 88% after 500 years (to 38.8%). EINPW and YK maintain similar proportions of their existing diversity (87% and 85% after 200 years, and 79% and 77% after 500 years;

Figure 7), resulting in heterozygosities of 45.6% and 41.1% for EINPW, and 40.8% and 37.0% for YK. HLWBRP and NQ maintain the smallest proportions of their diversity (75% and 52% after 200 years, and 53% and 35% after 500 years; Figure 7), resulting in 41.0% and 28.0% heterozygosity for HLWBRP. As the current heterozygosity of NH and NQ are unknown, estimates of future heterozygosities cannot be made for these populations. Overall, when considered as a panmictic metapopulation, wood bison lose diversity at a rate similar to that of WBNP (Figure 7).

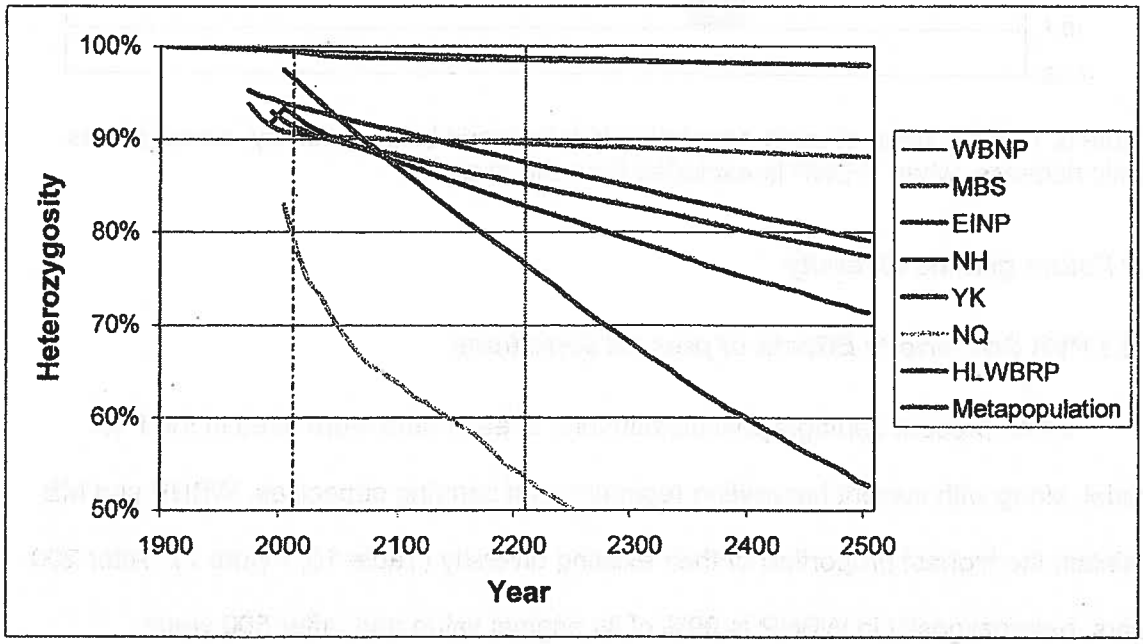

Figure 7. The change in heterozygosity in individual wood bison populations and the entire metapopulation. The dashed line represents the estimated proportion of heterozygosity in 2004. The solid line represents the estimated proportion of heterozygosity after 200 years.

Table 15. Herd size, carrying capacity, and projected heterozygosity of wood bison populations.

Herd	Current Size	Carrying Capacity	Existing He	Projected Percent of Existing He		Projected He	
				200 years	500 years	200 years	500 years
WBNP	4495	4500	55%	99%	98%	54.4%	53.8%
MB	2000	2000	44%	89%	88%	39.4%	38.8%
EINPW	320	450	52%	87%	79%	45.6%	41.1%
NH	200	320 [a]	n/a	83%	71%	n/a	n/a
YK	530	530	48%	85%	77%	40.8%	37.0%
NQ	62	120 [a]	n/a	52%	35%	n/a	n/a
HLWBRP	120	125	53%	75%	53%	41.0%	28.0%
Metapopulation	7727	8040	51% [b]	99%	98%	51.0%	50.0%

[a] Estimate based on current size and expectation that NH and NQ will merge to form a herd of approximately 400.

[b] Calculated as mean heterozygosity for all populations where this value is known, weighted by total population sizes.

3.2.2 PVA Scenario 2: Effects of founding events

When individual herds were modeled independently, assuming unique alleles at the time of establishment, the loss of genetic diversity over time occurred at a slower rate than when herds were founded from WBNP or EINPW (Figure 8). When founding alleles were unique, remaining heterozygosity ranged from 70% (NQ) to 92% (MB and YK) after 200 years, and 47% (NQ) and 91% (MB) after 500 years. Remaining heterozygosity in HLWBRP would be 76% and 55% after 200 and 500 years.

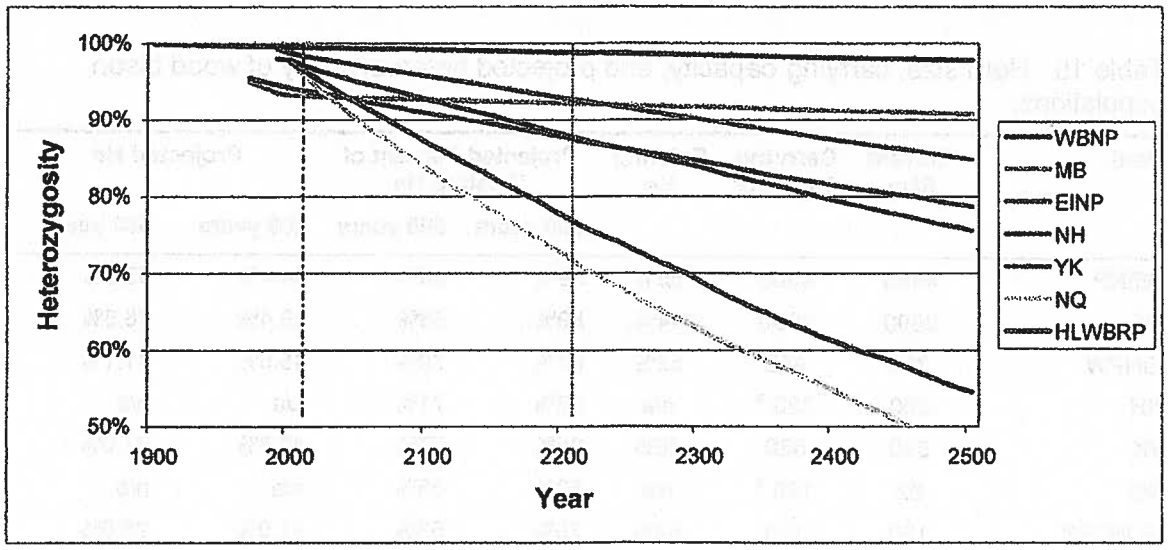

Figure 8. The change in heterozygosity in individual wood bison herds if no founding events had occurred and all alleles in the founding individuals are unique. The dashed line represents the estimated proportion of heterozygosity in 2004. The solid line represents the estimated proportion of heterozygosity after 200 years.

3.2.3 PVA Scenario 3: Effects of multiple herds

Long-term retention of genetic diversity within the wood bison metapopulation is somewhat related to the number of herds within the metapopulation (Figure 9). Over 500 years, the greatest level of heterozygosity is maintained (98%) when either seven or six (all but HLWBRP) herds are established. If only WBNP had been established, or if MB and EINPW were established as well, the loss of genetic diversity would also occur slowly, resulting in retention of slightly less than 98% of each herds' original heterozygosity levels. If WBNP is removed, the metapopulation loses genetic diversity more rapidly, retaining 96% of its original levels after 500 years.

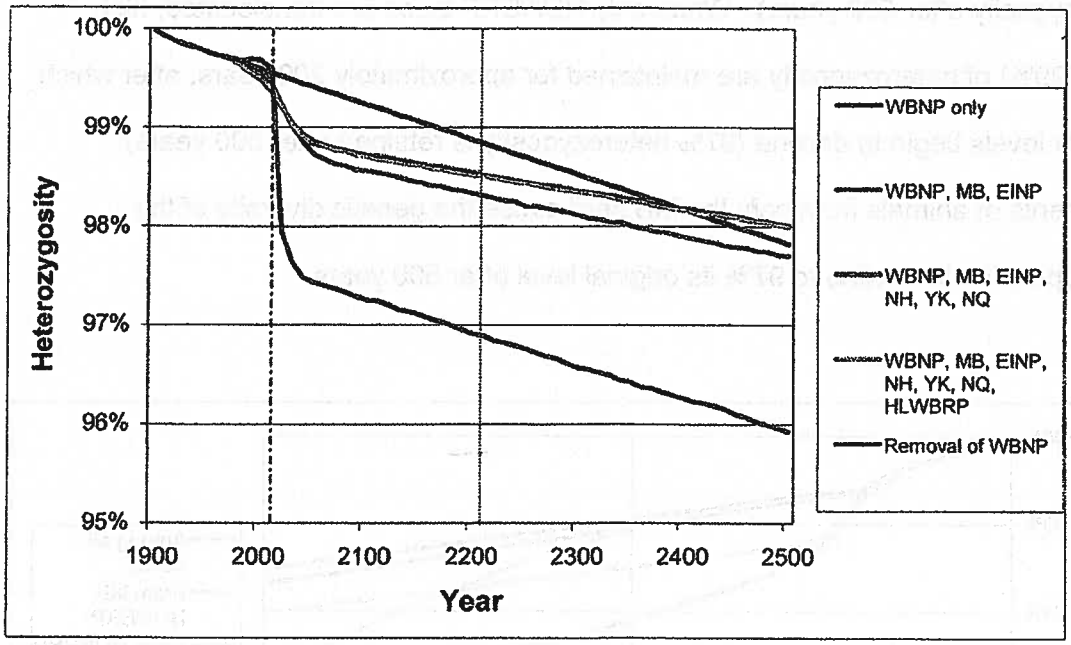

Figure 9. The change in metapopulation heterozygosity as herds are added or removed. The dashed line represents the estimated proportion of heterozygosity in 2004. The solid line represents the estimated proportion of heterozygosity after 200 years.

3.2.4 PVA Scenario 4: Effects of gene flow, regular culling, and additional salvage efforts

Movement of bison among herds will reduce the rate at which genetic diversity is lost from the metapopulation (Figure 10). The number of calves translocated each year also impacts the loss of genetic diversity; it occurs more slowly when 20 calves are moved annually from each herd, compared to 10 calves annually (Figure 11). The slowest rate of diversity loss occurs when gene flow occurs among all herds (99% heterozygosity is maintained after 500 years; Figure 10). Annual translocations of bison from only MB and HLWBRP retain similar levels of genetic diversity (98% heterozygosity after 500 years) to when animals are moved among all herds (99%

heterozygosity after 500 years). When only HLWBRP bison are translocated, high levels (99%) of heterozygosity are maintained for approximately 200 years, after which diversity levels begin to decline (97% heterozygosity is retained after 500 years). Movements of animals from only the MB herd cause the genetic diversity of the metapopulation to decline to 97% its original level after 500 years.

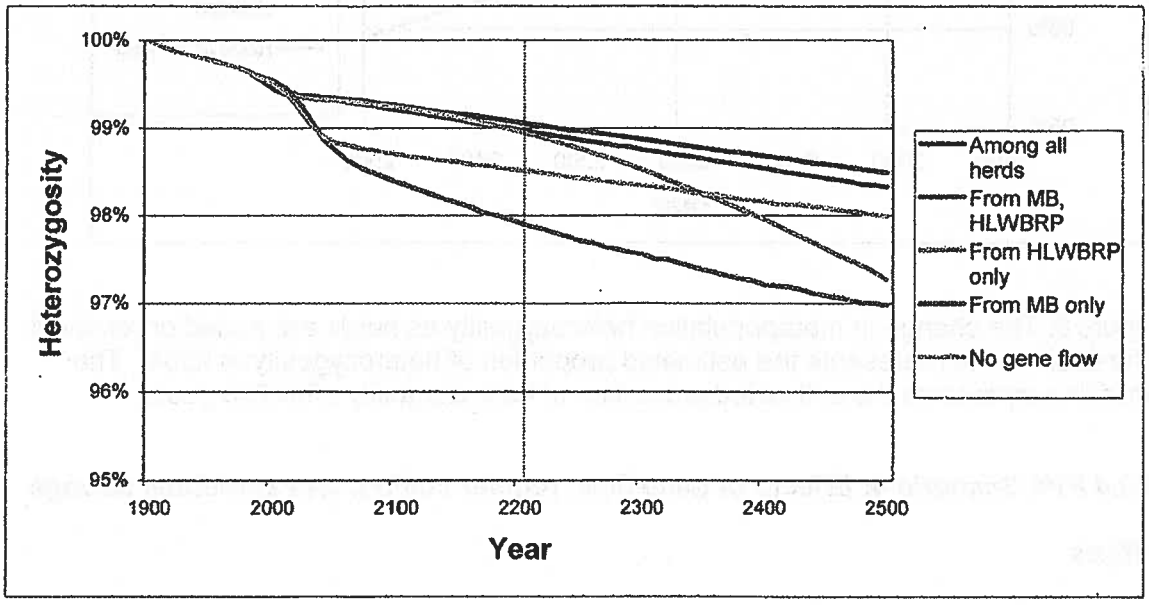

Figure 10. The change in metapopulation heterozygosity as annual translocations occur from MB and/or HLWBRP. Each year, 20 bison calves are moved from all herds (except WBNP), from MB and HLWBRP only, from HLWBRP only, and from MB only. Animals are never translocated into WBNP or HLWBRP. The solid line represents the estimated proportion of heterozygosity after 200 years.

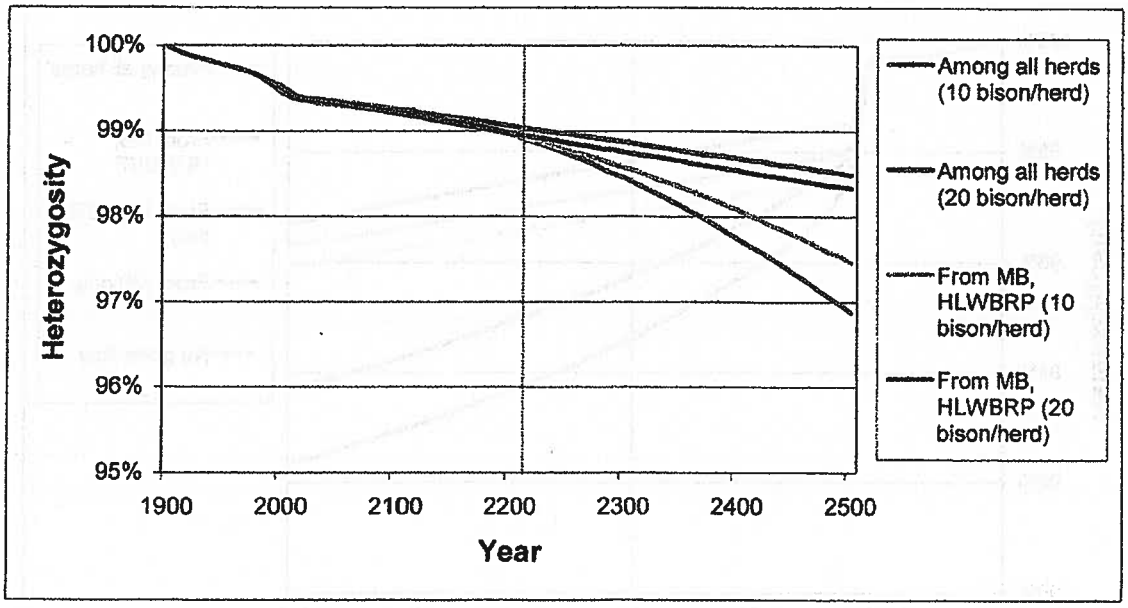

Figure 11. The change in metapopulation heterozygosity as batches of either 10 or 20 bison calves are moved annually. Translocations are modeled to occur from each herd (except WBNP) into all other herds, and from MB and HLWBRP into all herds. Animals are never translocated into WBNP or HLWBRP. The solid line represents the estimated proportion of heterozygosity after 200 years.

When WBNP is removed from the metapopulation, different trends are observed (Figure 12). The greatest amount of genetic diversity is retained when annual translocations occur among all herds (97% heterozygosity after 500 years), followed by when translocations occur from only HLWBRP (96% heterozygosity after 500 years). Movement of animals from only MB or from both MB and HLWBRP cannot retain diversity above the levels predicted if no gene flow were to occur.

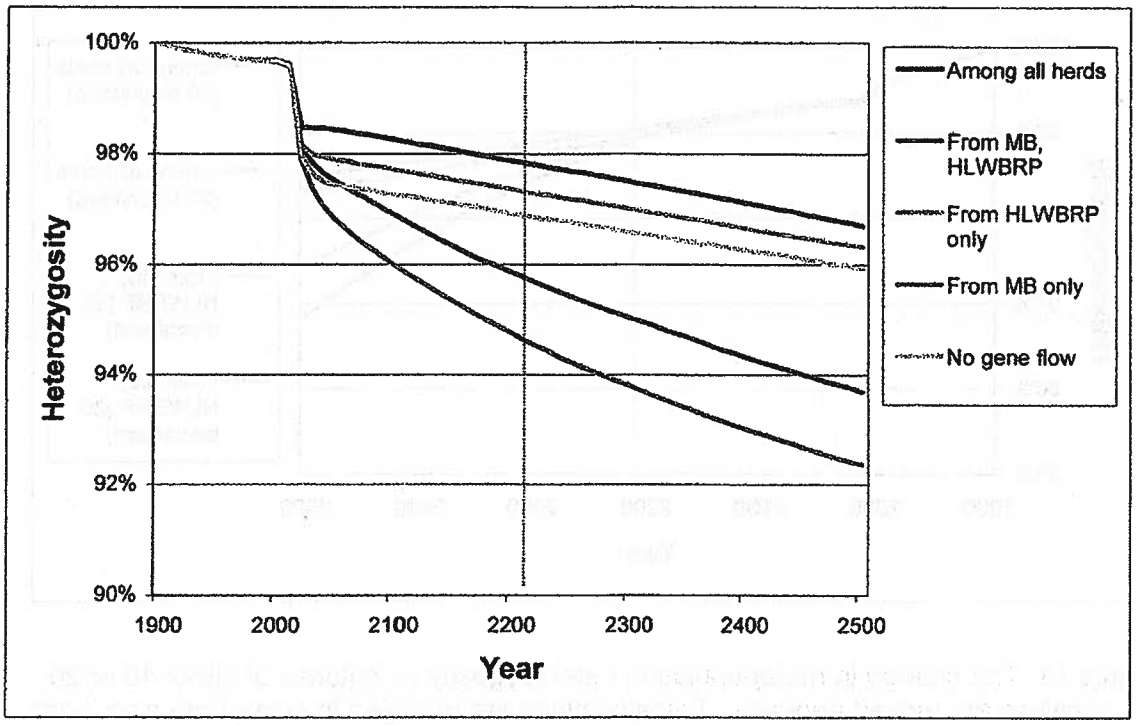

Figure 12. The change in metapopulation heterozygosity as annual translocations occur if WBNP were removed from the metapopulation without additional genetic salvage. This graph represents the heterozygosity in the metapopulation, independent of WBNP. After WBNP is depopulated, 20 bison calves are moved annually from all herds, from MB and HLWBRP only, from HLWBRP only, and from MB only. Animals are never translocated into WBNP or HLWBRP. The solid line represents the estimated proportion of heterozygosity after 200 years.

The genetic effects of moving animals only among the NH, YK, and NQ herds are negligible when WBNP is not removed from the metapopulation (Figure 13). Loss of genetic diversity is projected to occur at a rate comparable to when no gene flow is expected to occur, resulting in 98% heterozygosity after 500 years for both scenarios. Similar results are observed when bison calves are translocated from all herds into only NH, YK, and NQ, and when only bison from EINPW, MB, and HLWBRP are moved into NH, YK, and NQ (98% heterozygosity after 500 years) (Figure 13).

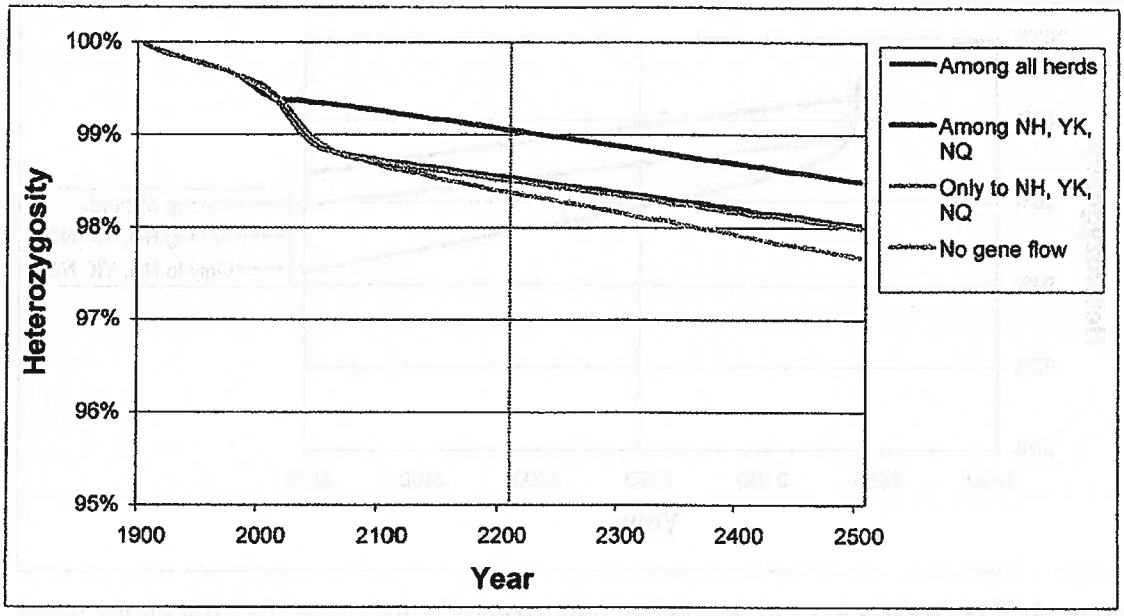

Figure 13. The change in metapopulation heterozygosity as annual translocations involving NH, YK, and NQ animals occur. Each year, 20 bison calves are moved among NH, YK, and NQ, and from all herds (except WBNP) into NH, YK, and NQ. Animals are never translocated into WBNP or HLWBRP. The solid line represents the estimated proportion of heterozygosity after 200 years.

When WBNP is removed, the metapopulation diversity is affected in a similar manner by annual translocations as when WBNP is present. Gene flow among all herds retains the highest proportion of genetic diversity (97% heterozygosity after 500 years; Figure 14). If bison from all herds are moved annually into only NH, YK, and NQ, genetic diversity declines at a faster rate (94% heterozygosity retained after 500 years) than if only bison from NH, YK, and NQ are involved in the annual movements (96% heterozygosity retained after 500 years).

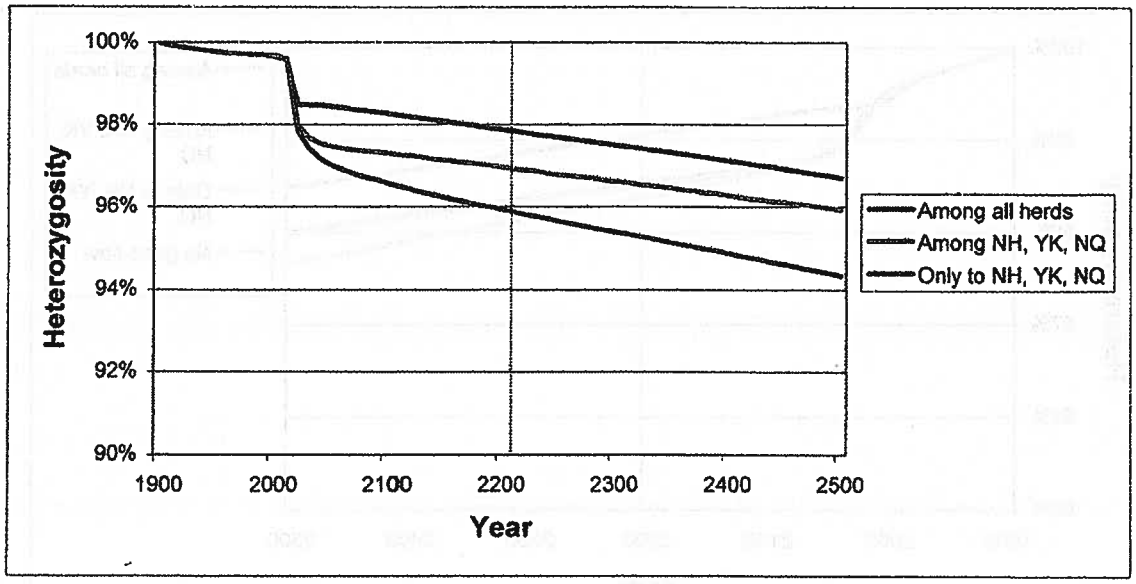

Figure 14. The change in metapopulation heterozygosity as annual translocations occur if WBNP is removed from the metapopulation without additional genetic salvage. This graph represents the heterozygosity in the metapopulation independent of WBNP. After WBNP is depopulated, 20 bison calves are moved annually among all herds, among NH, YK, and NQ, and from all herds (except WBNP) into NH, YK, and NQ. Animals are never translocated into WBNP or HLWBRP. The dotted line represents the estimated proportion of heterozygosity after 200 years.

Additional salvage attempts do not significantly contribute to the maintenance of genetic diversity of the metapopulation (Figure 15). When one and three new wood bison herds are established, the heterozygosity after 200 years and 500 years is approximately 99% and 98%, respectively. Genetic diversity is not significantly different between the two salvage scenarios. The same proportion of heterozygosity (98%) is retained after 500 years when additional salvage efforts are made as when the MB population is expanded to a carrying capacity of 2500.

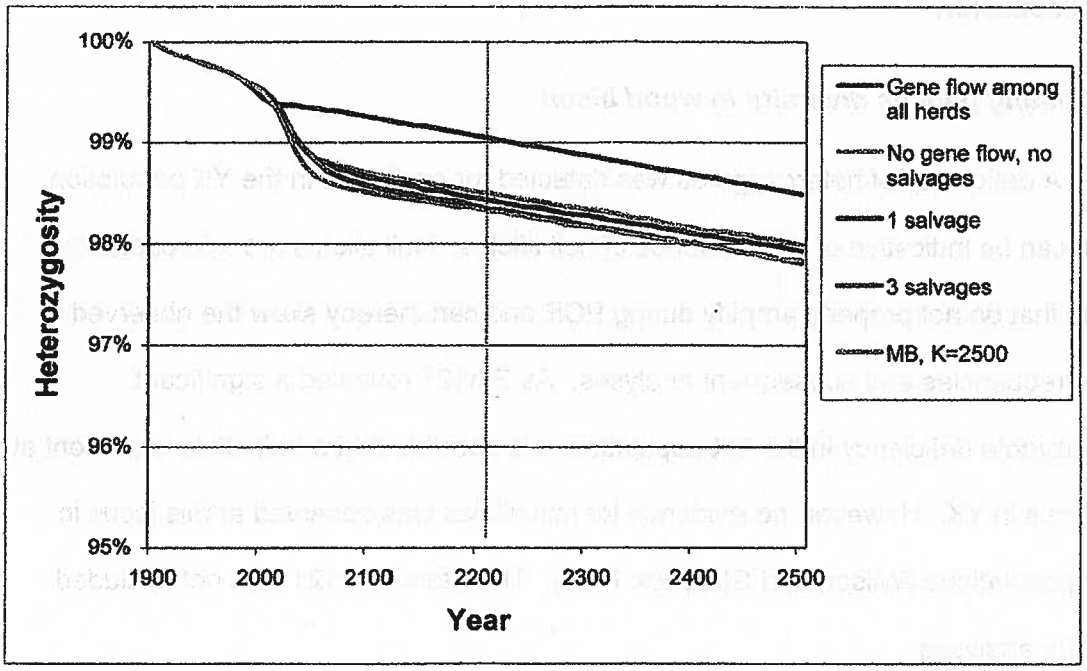

Figure 15. The change in metapopulation heterozygosity as additional salvage efforts are made, as annual translocations occur, and as the carrying capacity of MB is extended to 2500 bison. Salvaged herds are established with the same demographic distributions as the original HLWBRP population. No gene flow occurs when salvage efforts are made or when the carrying capacity of MB is extended. Animals are never translocated into WBNP or HLWBRP. The dotted line represents the estimated proportion of heterozygosity after 200 years.

4.0 Discussion

4.1 Existing genetic diversity in wood bison

A deficiency of heterozygotes was detected for one locus in the YK population, which can be indicative of the presence of null alleles. Null alleles are microsatellite alleles that do not properly amplify during PCR and can thereby skew the observed allele frequencies and subsequent analyses. As Eth121 revealed a significant heterozygote deficiency in the YK population, it is possible that a null allele is present at that locus in YK. However, no evidence for null alleles was observed at this locus in other populations (Wilson and Strobeck 1999). Therefore, Eth121 was not excluded from the analyses.

As shown in Table 3, YK is the least variable of all wood bison populations, based on most diversity measures. This is unsurprising as YK was founded from EINPW animals, which form one of the least variable wood bison populations. The number of cross-assignments between YK and EINPW (Table 5), the relatively small genetic distance between these populations (Table 6), and their grouping on the neighbour-joining tree (Figure 2), show that these two populations are fairly similar genetically. Their similarity is likely a result of the small number of founders for the YK population and the few generations separating the source population from the founding animals.

As shown in Wilson and Strobeck (1999), the WBNP and HLWBRP populations are the two most genetically similar populations, illustrated by the large number of cross-assignments between these populations (Table 5) and their proximate positioning on the tree (Figure 2). This is a more extreme example of the scenario observed

between YK and EINPW, as the HLWBRP population was established from a larger number of founders, and there has been little time for genetic drift to act on this population. Consequently, no population is more similar to WBNP than HLWBRP. In total, less than 2% of wood bison misassigned to a plains bison population. All of these were individuals from WBNP.

For all but two loci, RT24 and BM143, the observed numbers of alleles and expected heterozygosities in YK fell within the 95% C.I. simulated by GENELOSS. The heterozygosity and number of alleles at locus RT24 were below the 95% C.I., whereas diversity at BM143 was above the 95% C.I. Neither RT24 nor BM143 were loci for which an extended data set was available, and therefore initial estimates of diversity by GENELOSS were based on a smaller sample size. Consequently, the low diversity of RT24 can be explained by a sampling bias. Furthermore, the observed diversity in the YK population could be biased as only a small proportion of this population was collected for DNA sampling.

The large number of observed alleles and expected heterozygosities at BM143 could be explained by incomplete sampling in the EINPW population. More alleles were seen at locus BM143 in the YK population than in EINPW, the founding population. Three possible scenarios could explain this result; the new allele arose by mutation early in the YK population's history and rose to an appreciable frequency through genetic drift, it was present in the EINPW population but has since been lost, or it is still present in the EINPW population but was not sampled. Of these the last situation is the most likely, given the low rate of mutation, the probability of quickly losing an allele from

EINPW given its sample size, and the relatively small proportion of the EINPW population that were sampled at this locus.

Another factor potentially affecting the results of this simulation is that samples collected in 1998 in EINPW are being used to estimate those from 1986. It is possible that genetic drift over this time period would have altered the allele frequencies in EINPW. However, given the large size of this population and the small number of generations since that time, any changes would likely be minimal. Finally, it should be noted that while most of the observed genetic diversities fall within the 95% C.I., the standard deviations of the simulated values tend to be large (Tables 7, 8).

All simulated measures of diversity for the NQ population were less than those simulated for the YK population, owing to the consistently smaller number of reproductive pairs for NQ. The simulated number of alleles for the NQ population was smaller than that observed in any other bison population (Table 9). Simulated heterozygosity values for NQ were smaller than those seen in the YK population, except for the case of a 2-generation bottleneck of 8 reproductive pairs (Table 10). Thus, simulations suggest that the NQ population is one of the least, if not the least, genetically diverse wood bison population examined to date.

WBNP contains over 97% of the alleles observed in wood bison, and seven of the eight private alleles (Table 3). A sizeable proportion of wood bison genetic diversity is represented only in this herd. Both allelic richness and private alleles are dependant on sample size, however, and the largest samples were obtained from this population. Therefore, some of these alleles may exist in other populations, but remain unsampled. As expected from looking at other measures of diversity (e.g., heterozygosity and

probability of identity), the YK population contains the lowest proportion of the alleles found in wood bison. When WBNP is not considered in the analysis, HLWBRP became the population with the highest proportion of wood bison alleles (92%), and the largest number of private alleles (Table 4). The HLWBRP contained the largest amount of diversity found in WBNP, and was therefore a valuable resource of wood bison genetic material.

The genetic importance of the WBNP population to wood bison diversity is evident with both the expected heterozygosity and, especially, the allelic richness with rarefaction measures (Figures 3, 4; Tables 11, 12). Both of these measures are relatively unaffected by sample size. In both instances, the importance of WBNP is even greater when the contribution due to divergence is removed, leaving only the diversity contribution. This is a result of the relatively small genetic distances between WBNP and the other wood bison populations, particularly the HLWBRP (Table 6).

It may be essential to consider the contribution of divergence to genetic importance when populations share a small number of alleles, or if population divergence is a result of differential selection regimes. However, private alleles are generally uncommon in bison populations and founding events, rather than selection, largely control population relationships. Consequently, the diversity contribution is the primary factor that should be considered for genetic importance.

HLWBRP was the only other population to make a positive contribution to the diversity portion of genetic importance with both measures (Figures 3, 4; Tables 11, 12). However, the overall contribution of HLWBRP to heterozygosity was negative, due to the large negative contribution to the divergence portion of genetic importance. This

was likely a result of the close relationship between HLWBRP and WBNP, due to the recent founding event of HLWBRP from WBNP that included a large number of individuals. Also, genetic distances between HLWBRP and other populations tend to be smaller than those distances not involving HLWBRP or WBNP (Table 6).

To determine the importance of wood bison populations if WBNP is removed from the gene pool, genetic importance was recalculated without this population. Both EINPW and, slightly, MB had positive total genetic importance values when heterozygosity was considered (Figures 5, 6; Tables 13, 14). However, EINPW and HLWBRP were the populations that contributed positively to the diversity portion of heterozygosity. HLWBRP was the only population that positively contributed to the genetic importance of allelic richness values, and was joined by MB when only diversity was considered. Allelic richness is commonly described as the most relevant measure of genetic diversity (Schoen and Brown 1993, Bataillon *et al.* 1996, Petit *et al.* 1998). Consequently, if WBNP were removed from the wood bison metapopulation without additional genetic salvage, the HLWBRP would have become the most important source of genetic diversity.

4.2 Projected genetic diversity based on current management scenarios

Based on present demographic distributions, current harvesting regimens, and existing carrying capacities, the projected genetic diversity of each herd declined at a rate relative to the herd's population size (Figure 7). WBNP and MB are the largest herds (N = 4495 and N = 2000, respectively (M. Bradley and J. Nishi, pers. com. 2004)) and maintain the highest proportion of their existing diversity over 500 years (98% and 88% heterozygosity, respectively). EINPW (N = 320) and YK (N = 530) have similar

herd sizes and maintain similar proportions of their existing diversity after 500 years (79% and 77% heterozygosity). The smallest herds, NH (N = 200), HLWBRP (N = 120), and NQ (N = 62), maintain the smallest proportions of their diversity (71%, 53%, and 35% heterozygosity). Herds that can retain larger sizes are better able to maintain existing levels of genetic diversity, as the influence of genetic drift is inversely proportional to population size. Furthermore, density would not be as considerable a limiting factor of mean generation time for individuals belonging to populations on a large range.

Although population size has a significant influence on the ability of a herd to retain genetic diversity, loss of genetic diversity is also impacted by several other demographic factors. The number of founding events or bottlenecks experienced by a herd affects the initial level of diversity within that population (Figure 8). The WBNP population, which experienced only a single recent bottleneck, contains the highest levels of genetic diversity (Tables 3, 4). The EINPW, MB, and HLWBRP populations were founded from WBNP animals and contained relatively large levels of initial diversity. As HLWBRP was established from the largest group of WBNP founders, its initial diversity was high. In comparison, the NH, YK, and NQ populations, which were established from EINPW animals, experienced two founding events and could not contain more diversity than that present in EINPW at the time of their inception.

Using most measures, WBNP, EINPW, and HLWBRP had the highest levels of genetic diversity (Table 3). After 500 years, WBNP and EINPW are still expected to have the highest levels of diversity (Figure 7; Table 15). HLWBRP, on the other hand, was projected to rapidly lose diversity due to its small population size and carrying

capacity (and see Wilson *et al.*, 2005). Genetic management was necessary for the small HLWBRP herd to maintain high diversity in the long term. Consequently, given the operational and economic constraints for carrying capacity at the HLWBRP, we developed a captive breeding and genetic management plan to reduce the rate of loss in genetic diversity at the HLWBRP (Wilson *et al.* in prep).

Despite its larger size and consequent slower rate of diversity loss, MB will be less variable than EINPW after 500 years due to the current higher level of diversity at EINPW (Table 15). Both the MB and EINPW herds were founded from WBNP bison in the 1960s, but the number of founders for the EINPW population was slightly higher (Table 1). The stochastic processes of genetic drift since that time could also have had varying effects on the diversity within these populations.

Both NQ and NH are expected to lose diversity at a rate relative to their herd size. Simulations suggest NQ is among the least variable wood bison populations. Current levels of diversity for NH are unknown.

4.3 Contribution of herds to the future metapopulation diversity

Although the rates at which individual herds lose genetic diversity vary, the metapopulation maintains diversity at approximately the same rate as the WBNP herd (Figures 7, 9). As well as being the largest herd, comprising roughly 60% of the wood bison metapopulation, WBNP is also the founding population for all other wood bison herds and contains a significant proportion of the diversity within the metapopulation (Tables 11, 12). Depopulation of WBNP without salvage would significantly reduce the overall diversity of Canadian wood bison (Figure 9). However, depopulation with adequate genetic salvage would prevent or minimize this loss of overall diversity.

There is a slight inverse relationship between the number of herds in the metapopulation and the rate at which diversity is lost. As the contribution of WBNP to the metapopulation diversity is so large, the rate of diversity loss does not significantly differ between scenarios when only WBNP, or only WBNP, EINPW, and MB, or all but HLWBRP, or all seven herds are established. However, although values are similar, the rate at which diversity would be lost from a WBNP-only scenario is greater than that of a multiple herd scenario (Figure 9). It would therefore follow that the establishment of new herds should assist with the long-term conservation of genetic diversity, especially if WBNP is depopulated. Also, additional herds act as genetic banks, which could provide genetic material if any existing population is lost from the metapopulation.

4.4 Metapopulation management and projected genetic diversity

Periodic movements of animals among herds will significantly contribute to the overall diversity of the metapopulation. Our results suggest that establishing gene flow among all herds will be the best way to preserve the long-term genetic diversity of wood bison in Canada (Figures 10 -14). However, with respect to the contribution of unique genetic diversity, gene flow from HLWBRP, or MB and HLWBRP, would be most important (Figures 10, 12). This is because, aside from WBNP, all other herds are founded from EINPW animals and should be genetically similar to this population, although with lower levels of diversity. As such, they likely share a large amount of genetic diversity with one another, and movement of animals between these herds would not be the most efficient method of adding new genetic material to these populations. Translocations of animals from HLWBRP, or MB and HLWBRP, would provide new genetic material to herds founded by EINPW. Nonetheless, as populations

drift and become more dissimilar over time, moving animals from EINPW to other populations may increase metapopulation diversity. The movement of additional EINPW animals can also overcome the effects of founding events in cases where the number of founders was small and did not contain a representative amount of diversity.

Although movements of animals from both MB and HLWBRP could significantly contribute to long-term maintenance of diversity, translocation of animals from HLWBRP would have had the greatest effect (Figures 10, 12). Movements of animals solely from the MB herd will be insufficient for maximum retention of genetic diversity. This is likely due to the small founding size of the herd (N = 16). In comparison, due to its relatively large founding size (N = 57) and recent founding event, HLWBRP possessed the greatest amount of genetic diversity and would have therefore contributed more variation to the metapopulation (see Wilson *et al.*, 2005). From a theoretical perspective, annual gene flow from HLWBRP over a 200-year period would have contributed the most to overall metapopulation diversity (Figure 10). However, after 200 years of moving bison out of HLWBRP without bringing additional animals into the small herd, the genetic diversity of HLWBRP would decline drastically, likely as a result of genetic drift in this small population (Figures 10, 12). The overall diversity of the metapopulation will similarly decrease due, in part, to the loss of diversity from HLWBRP, as well as from the dilution of unique genetic material as animals are moved from a small, diverse population into large, less-diverse populations. These results reinforce the importance of maintaining HLWBRP at a sufficient size to preserve genetic diversity levels that are representative of the founding population.

The genetic effects of moving animals only among the NH, YK, and NQ herds are negligible because each of those herds were founded from similar genetic stock and currently have lower diversity than most other herds (Figures 13, 14; Table 15). Consequently, moving animals between these populations conserves a similar amount of genetic diversity as scenarios where no gene flow occurs. Furthermore, if gene flow were to occur from any herd, except WBNP, into only NH, YK, and NQ, genetic diversity will be lost at a faster rate than if no gene flow occurred (Figures 13, 14). This may be a result of the homogenization of genetic diversity in a number of populations as individuals from a less diverse source are moved between populations.

For the optimum preservation of genetic diversity for 500 years, translocations of bison among herds should involve as many animals as possible. For 200 years, the number of bison involved in the translocation does not significantly impact the rate at which diversity is lost (Figure 11). However, beyond 200 years, genetic diversity can be best maintained when 20 bison are moved from each herd annually, rather than only 10 bison from each herd annually. Movements of twice as many animals will incorporate a greater proportion of the existing genetic diversity into each herd. Although translocations occurred annually in our model, biannual movements of twice as many calves would be acceptable for preserving long-term genetic diversity. Moreover, biannual movements of twice as many calves may actually improve the retention of genetic diversity in the metapopulation, because entire cohorts could be removed together. This would not reduce the existing diversity of the source herd and adults could contribute their genetic material to the next generation during the following season. Unfortunately, this scenario cannot be tested using VORTEX. However, the

culling of calves biannually has been shown to be the most efficient method for maintaining diversity at ElNPW (Wilson and Zittlau 2004).

Regardless of the degree of gene flow established among herds, maintenance of large sizes will be important. Movement of animals should not occur if the herd size would be significantly reduced. Maintenance of herd size at approximately carrying capacity will minimize loss of diversity over time. However, without the influence of gene flow, an increase in carrying capacities of currently large herds will not significantly affect the metapopulation diversity (Figure 15). No change in the metapopulation diversity is observed when the carrying capacity of MB is increased from 2000 to 2500. In comparison, an expansion of the ranges of the smaller herds (e.g., NQ, NH, and HLWBRP) will likely have a considerable effect on the long-term preservation of diversity, particularly when gene flow is included. As this is not currently a viable option, the scenario has not been modeled.

Simulation results indicate that the establishment of one or three new herds provides no additional advantage to preserving genetic diversity of the overall wood bison metapopulation over the current scenario of no gene flow, so long as the WBNP population remains in existence (Figure 15). This is because WBNP currently has the greatest influence on metapopulation diversity, due to its large size. However, as recovery of wood bison is intrinsically tied to management of diseased populations (see Gates *et al.* 2001), additional salvage efforts will be essential for conserving and providing genetic material that is representative of WBNP, if diseased populations are to be removed from the metapopulation (see Shury *et al.* 2006).

Since each additional salvage attempt increases the ability of the metapopulation to maintain diversity, and the probability that a representative sample of the diversity within WBNP will be sampled, the establishment of new herds from WBNP will contribute significantly to the long-term conservation of diversity in disease-free wood bison. This is especially true if WBNP is to be depopulated. WBNP has the greatest genetic diversity of all wood bison herds. However, because bison in and around WBNP are infected with bovine tuberculosis and brucellosis, additional salvage efforts from these infected herds will require well-defined criteria, protocols, and health monitoring to establish successful eradication of these diseases. It will be important to conduct decision-making within an *a priori*, formalized, transparent risk-assessment process. This would allow managers to understand and balance the risk of disease transmission against the long-term benefits of increasing genetic diversity in bison populations (see APFRAN 2003, Lutze-Wallace *et* all 2006, Nishi *et al.* 2002a, 2002b, and 2004), and to provide an acceptable level of confidence that disease will not be introduced into healthy herds through management action.

Harvesting or culling also plays a significant role in the long-term conservation of genetic diversity. Previously, it was determined that, for EINPW, biannual removal of a group of calves and yearlings that represent 35% of the total herd size minimizes the loss of diversity over time (Wilson and Zittlau 2004). For other herds, optimal harvesting strategies from a genetic management perspective have yet to be determined. However, previous results suggest that regular culling of an equal number of young males and females can considerably slow the rate at which diversity is lost if a population is at carrying capacity. This occurs for several reasons. First, by removing

calf or yearling animals every second year, mature bison will persist in the population longer and have an increased opportunity to contribute to the gene pool before they die. Second, mature individuals that may not have successfully bred are less likely to be removed from the population. Finally, the mean generation time of the population will be increased because older individuals will be permitted to persist in the population longer. Since diversity is lost when individuals die, this can greatly reduce the population's loss of diversity.

As natural populations will undoubtedly change over time, management actions, such as the maintenance of habitat carrying capacities, regular translocations of calves, additional salvage efforts, and the targeting of young animals during biannual harvests, should be applied to test and evaluate the predictions made in this report at various time-points. It is critical to keep in mind that the predictions produced by the models examined in this report are dependant on both the assumptions of the model and the information supplied to the model. If these assumptions are found to be invalid, or the model parameters change dramatically, the predictions made by the model may no longer apply. Regular application and monitoring of recommended management actions will ensure that appropriate goals are still being targeted. Revisions to management priorities can be addressed as additional knowledge is acquired and alternative hypotheses are evaluated.

5.0 Conclusions

WBNP is the most genetically diverse population of wood bison, based on all measures. This population contains more unique diversity than any other population, and almost all wood bison diversity is represented within WBNP. As a result, WBNP is the most genetically important wood bison population, despite having low divergence values compared to other populations. If the diversity within this population were unavailable, HLWBRP would have become the most genetically important population, based on its allelic richness and the proportion of the remaining metapopulation diversity it would contain.

In most scenarios modeled, the wood bison metapopulation is able to maintain a suitable proportion of diversity over 500 years. The mean genetic diversity projected over time varies according to individual herd sizes, the number of herds in the metapopulation, and the degree of gene flow among herds. Population size has the most significant effect on the rate at which genetic diversity is lost. Maintenance of herd sizes at approximately carrying capacity (or a minimum population size of 400-500 or greater – see Wilson and Zittlau 2004) will best contribute to the long-term retention of genetic diversity.

In addition to large sizes, the number of herds among which gene flow occurs will significantly influence genetic diversity over time. The HLWBRP was an important herd due to its large number of founders, and high levels of genetic diversity that were nearly representative of WBNP. Gene flow from HLWBRP to other herds would have considerably reduced the rate at which diversity was lost. Maintenance of HLWBRP at as large a size as possible and management for diversity would have permitted long-

term future translocations from this herd without significantly impacting the HLWBRP diversity. If the HLWBRP was maintained at its carrying capacity and translocated animals were removed at random, genetic diversity would begin to decline more rapidly after 200 years due to the regular removal of bison without additions to the herd.

With the recent depopulation of the HLWBRP – due to the presence of bovine tuberculosis – the importance of additional salvage efforts is increased. Correspondingly, salvage of new herds from WBNP will be required in order to increase genetic diversity to the rest of the metapopulation. If established from appropriate numbers of founders, additional salvaged herds should also have high diversity that is representative of WBNP. Each additional salvage attempt will increase the diversity of the metapopulation.

The harvesting regimen of each herd will be an important consideration for managers. If a herd is at carrying capacity, regular culling or harvest of an equal number of young males and females should considerably reduce the loss of diversity over time. From a genetic conservation perspective, the removal of the youngest possible animals every second year would provide the best approach to maintaining diversity.

Population dynamics shift over time, so the management actions evaluated in this study should be monitored as new information is gathered and efforts are applied. Population simulations are dependant on model assumptions and the available population and species information. A change in the acceptance of any of these values can alter the expectations arising from a simulation. Consequently, new information

should be incorporated into new simulations when available and management strategies should be revised accordingly.

6.0 Recommendations

In this study, we incorporated genetic data with demographic data from seven wood bison herds in Canada. By combining current knowledge with simulated future changes in genetic diversity, we identified potential management strategies and evaluated the impacts of these strategies on genetic diversity over time. Based on the direction and severity of these impacts, several management strategies are recommended.

1. As the wood bison population at WBNP is the most genetically important for metapopulation diversity, further genetic salvage should be performed from WBNP to ensure the short- and long-term conservation of genetic diversity is managed in disease-free populations. If salvage is based on capturing live animals, genetic management (i.e., determination of the number of founders and breeding management) should reflect the technical knowledge gained from the HLWBRP. The goal of genetic salvage should be to sample high levels of genetic diversity that are representative of WBNP, with an appropriate number of founders. With every additional herd established, the diversity of the metapopulation will be increased. A useful strategy for genetic salvage of disease-free herds from the infected populations in and around WBNP would be to establish individual populations with large numbers of founders (i.e., $\geq$ 50). This should be followed by a rapid growth phase through monitored and/or managed breeding to a population size of at least several hundred.
2. The genetic importance of populations should be considered when choosing founding animals for newly established populations. Those with high importance should be given precedence as salvage sources.

3. The HLWBRP was one of the most genetically important populations, and based on the premise that it was disease-free, it was considered the most important healthy source herd for translocations. However, the confirmation of tuberculosis in the herd (June 2005) and the subsequent depopulation of the HLWBRP (March 2006), re-emphasizes the difficulty, importance, and need for additional genetic salvage. Salvage projects such as the HLWBRP require proper breeding and genetic management, because due to their small size, diversity can be lost quickly.
4. Larger wood bison herds (i.e., a census population size of several thousand) lose less genetic diversity over time. Therefore conservation herds should be maintained at their approximate carrying capacity to minimize loss of diversity. Over meaningful time, herd size is the most significant aspect affecting genetic diversity. Therefore, if the carrying capacity of any herd could be increased, loss of genetic diversity will be slowed accordingly. This is especially true for the smaller herds (i.e., less than a few hundred).
5. Gene flow among all healthy herds will significantly reduce the rate at which diversity is lost. Annual movements of 10 male calves and 10 female calves are recommended, although biannual movements of 20 male calves and 20 female calves are likely to have equivalent effects on the retention of genetic diversity.
6. If herd reductions are required, biannual removals of an equal number of young males and females will significantly slow the loss of genetic diversity over time. Herds should be at carrying capacity before regular removals are initiated.

7.0 Acknowledgments

Hal Reynolds (Canadian Wildlife Service) provided extensive administrative and technical support for this project, and provided helpful comments on previous manuscript versions. Wes Olsen and Mark Bradley (Parks Canada Agency) provided pertinent information on bison herds in Elk Island National Park and Wood Buffalo National Park respectively. Michelle Oakley and Philipp Merchant (Government of Yukon) collected and provided tissue samples from Aishihik herd in the Yukon. Jennifer Bonneville conducted DNA extractions and PCR analyses in the laboratory of Curt Strobeck at the University of Alberta, Edmonton, AB. Funding for this study was provided by the Interdepartmental Recovery Fund (Department of Environment, Canadian Wildlife Service), and the Department of Resources, Wildlife and Economic Development (South Slave Region, Government of the Northwest Territories).

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Journal of Heredity 2007:98(1):1–12
doi:10.1093/jhered/esl051
Advance Access publication December 16, 2006

A Comprehensive Evaluation of Cattle Introgression into US Federal Bison Herds

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Abstract

Genetic introgression, especially from interspecies hybridization, is a significant threat to species conservation worldwide. In this study, 11 US federal bison populations were comprehensively examined for evidence of both mitochondrial and nuclear domestic cattle (*Bos taurus*) introgression. Mitochondrial introgression was examined using established polymerase chain reaction methods and confirmed through analysis of D-loop sequences. Nuclear introgression was assessed in 14 chromosomal regions through examination of microsatellite electromorph and sequence differences between bison and domestic cattle. Only one population was identified with domestic cattle mitochondrial DNA introgression. In contrast, evidence of nuclear introgression was found in 7 (63.6%) of the examined populations. Historic accounts of bison transfers among populations were corroborated with evidence of introgressed DNA transmission. While neither nuclear nor mitochondrial domestic cattle introgression was detected in bison from Grand Teton National Park, Sully's Hill National Game Preserve, Wind Cave National Park, or Yellowstone National Park, adequate sample sizes were available only from the last 2 populations to allow for statistical confidence (>90%) in nuclear introgression detection limits. The identification of genetically unique and undisturbed populations is critical to species conservation efforts, and this study serves as a model for the genetic evaluation of interspecies introgression.

Near the apex of the decline of North American bison (*Bison bison*) in the late 1800s, a small number of individuals independently and effectively served to save the species from near-extinction by capturing and raising wild bison on 5 private ranches (Coder 1975). Nearly all bison that exist today are descendants of the less than 100 bison used to found these 5 private herds and a remnant wild population in Yellowstone National Park (YNP) of approximately 30 bison (Garretson 1938; Meagher 1973; Coder 1975). Bison produced in the private herds were used to establish public populations in the United States of America and Canada (Table 1), to which the lineages of the more than 500 000 North American bison in existence today can be traced. Therefore, federal and state bison populations in North America are a critical resource for long-term species conservation.

Hybrids are known to form among nearly all combinations of species from the *Bos* genus (van Gelder 1977), and molecular techniques have been used to assess the extent of nuclear introgression due to hybridization among some members of the genus (Davis et al. 1988; Nijman et al. 1999). Although generally considered to be from different but closely related genera, bison and domestic cattle (*Bos taurus*) can produce fertile offspring from human-controlled crosses (Jones 1907; Boyd 1908, 1914; Goodnight 1914). The 2 species are not known to produce hybrids naturally, and even carefully controlled crosses result in a low birth rate of viable first-generation hybrid offspring (Boyd 1908; Steklenev and Yasinetskaya 1982). Each of the ranchers involved in establishing the 5 bison foundation herds in the late 1800s either experimented with domestic cattle–bison crosses or purchased bison from others who were involved in such experiments (Garretson 1938; Coder 1975). Consequently, both mitochondrial (Polziehn et al. 1995; Ward et al. 1999) and nuclear (Halbert et al. 2005) evidence of domestic cattle introgression has been identified in both public and private bison populations. In a previous study, 14 unlinked microsatellite markers with nonoverlapping allele size ranges between bison and domestic cattle were used to identify bison populations with evidence of nuclear domestic cattle introgression; regions of introgression were subsequently confirmed through analysis of microsatellites closely linked to the original diagnostic loci (Halbert et al. 2005). To date, evidence of mitochondrial or nuclear domestic cattle introgression has been identified in all except 6 of 14 US and Canadian public bison populations (Ward et al. 1999; Halbert et al. 2005) and all except 1 of the more than 50 private bison herds examined to date (Derr JN, unpublished data).

The apparent success of the bison recovery efforts over the past 150 years is threatened by domestic cattle introgression. Hybrid species do not have taxonomic status and are not protected by the Endangered Species Act (O'Brien and Mayr

Table 1. History of establishment for 11 US federal bison populations, derived from Halbert (2003)

Herd	Location	Year	Founding stock (number, source)[a]
BNP	South Dakota	1963	3, FN; 50, TR (TRS)
		1983	20, Colorado National Monument (unknown origin)
FN	Nebraska	1913	6, private ranch, Nebraska; 2, YNP
		1935	4, CSP, South Dakota
		1937	4, CSP, South Dakota
		1952	5, NBR
GT	Wyoming	1948	20, YNP
		1964	12, TR
NBR	Montana	1908	1, Goodnight herd; 3, Corbin (McKay–Alloway); 34, Conrad (Pablo–Allard)
		1939	2, 7-Up Ranch (unknown origin)
		1952	4, FN
		1953	2, YNP
		1984	4, Maxwell State Game Refuge, Kansas (Jones)
NS	Iowa	1996	8, FN; 8, WM
		1997	6, FN; 8, NBR
		1998	3, FN
SUH[b]	North Dakota	1919	6, Portland City Park, Oregon (unknown origin)
		1932	1, WC
		1941–1979	7, FN
		1987	3, NBR
		1994–1997	2, TR
TR	North Dakota	1956 (1962)	29, FN to found south unit (TRS) [20, TRS bison to found north unit (TRN)]
WM	Oklahoma	1907	15, New York Zoological Park[c]
		1940	2, FN
WC	South Dakota	1913	14, New York Zoological Park[c]
		1916	6, YNP
YNP	Wyoming, Idaho, Montana	1902	Approximately 30 indigenous; 18, Pablo–Allard herd; 3, Goodnight herd

BNP, Badlands National Park; FN, Fort Niobrara National Wildlife Reserve; GT, Grand Teton National Park; NBR, National Bison Range; NS, Neal Smith National Wildlife Reserve; SUH, Sully's Hill National Game Reserve; TR, Theodore Roosevelt National Park; WM, Wichita Mountains National Wildlife Reserve; WC, Wind Cave National Park; YNP, Yellowstone National Park.

[a] Five private foundation herds established in the late 1800s (Coder 1975): McKay–Alloway (Canada), Goodnight (Texas), Dupree–Philip (South Dakota), Jones (Kansas), and Pablo–Allard (Montana).

[b] History of introductions provided by Dixon C (personal communication).

[c] As described by Coder (1975); founded as composite of bison from Nebraska (1888), South Dakota (1889), the Pablo–Allard herd (1897), and the Corbin herd (1904), which originated from bison from Wyoming, Manitoba, and the Jones herd.

1991). Widespread hybridization in other mammalian species has lead to proposals to delist such icons as the red wolf and Florida panther as endangered species (Rhymer and Simberloff 1996). However, bison have at least 2 advantages to successful long-term conservation over other partially introgressed species: a large total population size (>500 000 bison in existence today) and many moderately sized, isolated, and protected public populations (census sizes >200). As such, the purpose of this study was to comprehensively examine bison from US federal populations for evidence of both mitochondrial and nuclear domestic cattle introgression to identify potentially important sources of germplasm for long-term species conservation efforts.

Materials and Methods

Sample Collection and DNA Isolation

Bison blood, hair, or tissue samples were collected from 11 US federal populations (Table 2) by park personnel. DNA was extracted from whole blood following the Super Quik-Gene protocol (Analytical Genetic Testing Center, Denver, CO) and standard phenol–chloroform–isoamyl alcohol extraction (Sambrook et al. 1989) or isolated through application to FTA cards and processing following the manufacturer's recommendations (Whatman, Newton Center, MA). DNA was extracted from hair follicles and tissues following the protocols by Schnabel et al. (2000) and Halbert et al. (2004), respectively, and archived at Texas A&M University for future reference.

Mitochondrial DNA Introgression Assay

All polymerase chain reaction (PCR) and sequence reactions were performed on GeneAmp PCR System 9700 thermal cyclers (PE Biosystems, Foster City, CA). The mitochondrial DNA (mtDNA) assay was as described by Ward et al. (1999) with minimal exceptions as follows (per 25 µl reaction): 50 ng template DNA or 1 FTA punch, 0.2 µM each primer, 1× MasterAmp PCR Enhancer (Epicentre, Madison, WI), 400

Table 2. Total number of bison examined among 11 federal populations for mitochondrial and nuclear domestic cattle introgression

Population	Collection year	Census[a]	Total sampled
BNP	2002	875	492
FN	2001–2002	380	367
GT	1999–2000	600	39
NBR	1999–2002	350	616[b]
NS	2001	63	63
SUH	2004	35	31
TRN	2000	312	294
TRS	2001	371	355
WM	1999, 2002	600	172
WC	1999–2001	350	352[b]
YNP	1997, 1999–2002	3000	520
Sum		6936	3301

[a] Current approximate census population size, as estimated by individual herd managers. When possible, estimates are given of total census population size at the time of collection for this study (or average across collection years).

[b] Total sampled greater than given census size due to sampling of all adults in a population and calves over multiple years. Duplicate samples from the same individual were eliminated from analysis through comparisons of unique collection identifiers (ear tags, microchips) or polymorphic microsatellite analysis (Halbert 2003).

μM deoxynucleoside triphosphate (dNTPs), 2.0 mM $MgCl_2$, 1× reaction buffer, and 1.0 units *Taq* DNA polymerase (Promega, Madison, WI). The thermal parameters for the mtDNA assay were as follows: 96 °C for 3 min; 4 cycles of 96 °C for 20 s, 58 °C for 30 s (−1 °C per cycle), and 65 °C for 90 s; 26 cycles of 96 °C for 20 s, 54 °C for 30 s, and 65 °C for 90 s; and 1 cycle of 96 °C for 60 s, 54 °C for 60 s, and 65 °C for 20 min.

Sequencing of the mtDNA D-loop was performed for bison with suspect domestic cattle fragments. An 1100-bp fragment was amplified using the primers 12S (5′-AACAGGAAGGCTGGGACC-3′) and THR (5′-AGAGAAGGAGAACAACTAACCTCC-3′) located in the 12S rRNA and threonine tRNA genes, respectively, flanking either side of the bovine D-loop. Amplification was performed under the following conditions (per 50 μl reaction): 100 ng template DNA, 0.12 μM each primer, 400 μM dNTPs, 3.5 mM $MgCl_2$, 1× reaction buffer, and 1.25 units AmpliTaq Gold® DNA polymerase (PE Biosystems). PCR products were cleaned using the QIAquick PCR Purification Kit (Qiagen Inc., Valencia, CA). Sequence reactions were performed using the BigDye® terminator cycle sequencing kit version 2.0 (PE Biosystems) and an ABI377 automated sequencer (PE Biosystems) with the THR and internal D811-R (770 bp from THR, 5′GGGGGAATTTTTATGGAGG-3′) primers.

Sequences obtained in this study were compared with those produced by Ward et al. (1999) using CLUSTALX (Higgins and Sharp 1988) with the following alignment parameters: gap opening of 15, gap extension of 6.66, and transition weight of 0.5. Phylogenetic Analysis Using Parsimony (PAUP* 4.0b2; Swofford 2003) was employed for parsimony analysis of the sequences through branch-and-bound algorithms with the following options: unrooted starting trees obtained via stepwise addition, tree-bisection-reconnection used as the branch-swapping algorithm, branches collapsed when maximum length equals zero, and bootstrapping on a 50% majority rule consensus tree with 2000 replicates to test the strength of relationships among taxa.

Nuclear Introgression Assay

One marker from each of the regions examined by Halbert et al. (2005) was chosen for examination in this study based on the presence of introgression in other bison populations screened (Halbert et al. 2005) and allele size ranges for multiplexing. The forward primer for each marker was fluorescently labeled and multiplexed according to nonoverlapping allele size ranges and dye types (Table 3). All microsatellite amplification reactions were performed using the thermal parameters described above for the mtDNA assay. PCR conditions for multiplexes A and C and all confirming markers were as follows (5 μl reactions): 50 ng template DNA or 1 FTA punch, 0.05–0.4 μM each primer, 1× MasterAmp PCR Enhancer (Epicentre), 400 μM dNTPs, 3.0 mM $MgCl_2$, 1× reaction buffer, and 0.375 units *Taq* DNA polymerase (Promega). PCR conditions for multiplex B were as above with the exception of 1.6× reaction buffer.

All 14 nuclear diagnostic microsatellites were screened across all samples. Markers were rerun as singletons in individuals with suspect domestic cattle–like alleles using essentially the same PCR protocols as above, with water substituted for the extra primer volume. For those populations with suspect domestic cattle–like alleles at a diagnostic locus, bison were divided into 2 classes: those with domestic cattle–like alleles and those with exclusively bison alleles. At least one linked confirming microsatellite (Table 3) was amplified in a singleplex PCR on a subset of each class, with all bison in the former class screened when possible. All PCR products were separated on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) using an internal size standard (Mapmarker LOW; Bioventures, Inc., Murfreesboro, TN). GENOTYPER 3.6 (Applied Biosystems) was used for allele identification and comparison.

Sequence Confirmation of Introgression

For each diagnostic microsatellite marker in which domestic cattle introgression was detected in one or more bison populations, representative alleles were sequenced from bison and cattle through a second PCR, TOPO TA Cloning® (Invitrogen, Carlsbad, CA) and subsequent sequencing following the manufacturer's recommendations. Briefly, isolated white colonies were grown in Luria–Bertani broth containing 50 μg/ml ampicillin. A 1-μl aliquot of the broth was added to 9 μl sterile water and denatured at 100 °C for 10 min. Immediately after denaturation, the samples were placed on ice and used as template for standard microsatellite PCR amplification and allele detection as described above. After determining the alleles represented in each clone, plasmid preparations were obtained and inserts sequenced for clones representing each of the following allele classes: bison allele, domestic cattle–like allele identified in bison, and domestic cattle allele of the same size (electromorphs) from

Table 3. Primary diagnostic and closely linked confirming microsatellite markers (in italics) for 14 chromosomal regions used to detect nuclear domestic cattle introgression in bison

Locus	Label[a]	Multiplex	Chromosome	Position[b]	*Bison bison* allele range[c]	*Bos taurus* allele range[d]
AGLA17	VIC	A	1	0	215	214–219
BM4307	6-FAM	C	1	35.2	185–187	183–199
BMS4017	HEX		1	34.8	145–165	148–158
BM7145	NED	A	1	69.2	108–110	116–118
INRA119	HEX		1	68.7	122–128	130–138
BMS4008	6-FAM		1	71.7	158–164	152–179
BMS4040	NED	B	1	98.8	75, 95[e]	85–99
CSSM42	NED	B	2	34.4	167–171	173–217
AGLA293	HEX	C	5	32	218–220	218–239
RM500	6-FAM	A	5	55.6	123	125–135
SPS113	VIC	A	10	29.2	128–132	135–154
BM4513	NED	A	14	62.5	132–134	139–166
TGLA227	VIC	B	18	84.7	73	79–106
RM185	HEX	C	23	45.1	92	90–108
BMS2270	6-FAM	A	24	21.2	66–70	80–98
ILSTS065	HEX		24	25.2	Null[f]	131–143
BM1314	6-FAM	B	26	24.8	137	143–167
HEL11	6-FAM		26	20.7	142–175	179–203
CSSM36	VIC	A	27	39.8	158	162–185

[a] Fluorescent dye label for forward primer (Applied Biosystems).

[b] Chromosomal position (cM) as reported in the USDA cattle gene mapping database (www.marc.usda.gov).

[c] Based on the YNP and WC populations in this study and the results of Halbert et al. (2005).

[d] Based on the results of Halbert et al. (2005) from 64 domestic cattle (10 Angus, 16 Hereford, 13 Holstein, 12 Shorthorn, 13 Texas Longhorn).

[e] The 95-bp BMS4040 allele was found by Halbert et al. (2005) only in the CSP population and presumed to be of bison origin based on the exclusive presence of bison-like alleles at a nearby locus.

[f] ILSTS065 does not amplify in bison due to the presence of a fixed null allele.

domestic cattle. Multiple clones containing the same allele were sequenced to resolve any suspect sequence anomalies. Preliminary alignments were established using the program CLUSTALX (Higgins and Sharp 1988) as described above, which were then checked manually and adjusted as necessary around the repeat regions. Repeat region length differences and single nucleotide polymorphisms were recoded in a 1–0 matrix containing all the alleles sequenced. PAUP* 4.0b2 (Swofford 1999) was used to establish relationships among alleles as described above.

Statistical Analysis

The statistical model outlined by Halbert et al. (2005) was used to estimate the probability of detecting domestic cattle introgression in the bison populations examined in this study as follows: assume 2 categories of founders for a given bison population, hybrid founders and purebred founders, and let p be the expected proportion of haploid domestic cattle genome represented in the hybrid founders such that an F_1 (first-generation cross) hybrid as a founder would represent the entire domestic cattle genome ($p = 1$) and a BC_1 (first-generation backcross) hybrid as a founder would represent half the domestic cattle genome ($p = 0.5$). Assume then that the hybrid founders are merged with a group of purebred bison and allowed to randomly mate for a sufficient number of generations such that each bison within the population has some proportion, m, of nuclear domestic cattle introgression. In a random sample of n individuals and using t independent, selectively neutral, unlinked diagnostic markers to detect introgression, a marker is considered informative for detecting introgression if it falls into the region of the genome for which domestic cattle DNA was present in the hybrid founders. Therefore, the probability of detecting introgression within a population is represented by

$$P(p, m, n, t) = 1 - \left[p\left(1 - \frac{m}{p}\right)^n + (1 - p)\right]^t, \quad (1)$$

for $m \leq p$.

Results

A total of 3301 bison from 11 federal populations were surveyed for evidence of domestic cattle introgression using both mitochondrial and nuclear loci (Table 2). Sampling of all or nearly all bison from individual populations was performed when possible but was not achieved for the Badlands National Park (BNP) (56.2%), Grand Teton National Park (GT) (6.5%), Wichita Mountains National Wildlife Refuge (WM) (28.7%), and YNP (17.3%) populations.

Of the 11 federal populations examined, evidence of domestic cattle mtDNA introgression was found only in bison from National Bison Range (NBR), where suspect cattle D-loop fragments amplified in 11 of 616 tested bison (1.8%). Of these, 2 were females (born in 1984 and 1989) and 9 were males (1 each born in 1989, 1994, 1998, 1999, and 2000 and 3 born in 2002; 1 of unknown age). D-loop sequencing was performed for 8 of the suspect bison, excluding the 3 males born in 2002. Sequence alignments revealed

Table 4. Summary of testing and results for confirming loci by population

			DL suspect			DL nonsuspect		
Population	DL	CL	Domestic cattle allele (CL)	CL tested	CL cattle allele	Bison alleles (CL)	CL tested	CL bison allele
WM	BM1314	HEL11	187	7	7	155, 159, 161	7	7
BNP	BM4307	BMS4017	154	123	121	155, 159, 161, 163	366	366
FN	BM4307	BMS4017	154	73	69	155, 159, 161, 163	211	211
NS	BM4307	BMS4017	154	16	15	153, 155, 159, 161, 163	46	46
TRN	BM4307	BMS4017	154	91	90	155, 159, 161, 163, 165	210	210
TRS	BM4307	BMS4017	154	76	76	153, 155, 159, 161, 163	289	289
NBR	BM7145	INRA119	132	45	45	124, 126, 128	12	12
		BMS4008	166	45	44	160, 162	11	10
NS	BM7145	INRA119	132	2	2	124, 128	3	3
		BMS4008	166	2	2	160, 162	3	3
BNP	BMS2270	ILSTS065	131	30	14	Null[a]	12	12

Results presented only for those populations with suspect cattle-like alleles at DLs. Bison from each population were divided into 2 groups based on their DL genotypes: suspect (possessing cattle-like allele) or nonsuspect (possessing only bison-like alleles). Bison from each group were genotyped for the appropriate linked CL. The domestic cattle and bison called allele sizes for each CL in each population are indicated (following Halbert et al. 2005). DL, diagnostic locus; CL, confirming locus; CL tested, total number of bison tested in each class for the appropriate CL; CL cattle allele, the total number of tested bison with at least one cattle-like allele at the confirming locus; CL bison allele, the total number of tested bison with exclusively bison-like alleles at the confirming locus.

[a] ILSTS065 does not amplify in bison due to the presence of a fixed null allele.

complete identity to the domestic cattle mtDNA haplotype (9*) found in NBR bison by Ward et al. (1999). Likewise, parsimony analysis produced a consensus tree similar to that detailed by Ward et al. (1999), with the domestic cattle haplotypes from NBR bison sharing a node with domestic cattle of various breeds and other haplotypes identified as resulting from bison–domestic cattle introgression.

Allele frequencies for each of the 14 diagnostic microsatellites utilized for the detection of domestic cattle introgression are shown in Table A1 by population, with comparative frequencies for 5 domestic cattle breeds (n = 64 total) also shown (Halbert et al. 2005). Suspect domestic cattle–like alleles were detected at 4 of the 14 diagnostic microsatellites as follows: WM—9.01% frequency of BM1314 157-bp allele; BNP—13.55%, Fort Niobrara National Wildlife Refuge (FN)—13.48%, Neal Smith National Wildlife Refuge (NS)—13.49%, Theodore Roosevelt National Park north unit (TRN)—16.26%, and Theodore Roosevelt National Park south unit (TRS)—11.51% frequency of BM4307 197-bp allele; NBR—3.83% and NS—1.59% frequency of BM7145 116-bp allele; and BNP—3.15% frequency of BMS2270 94-bp allele (Table A1). In each population where potential domestic cattle introgression was detected at a diagnostic locus, confirmation of domestic cattle introgression was obtained through the detection of domestic cattle alleles at one or more linked loci (Table 4). In some cases, a small number of bison had a cattle-like allele at one locus but not at the other, indicating recombination (e.g., NBR BM7145/BMS4008; Table 4). One notable exception was the BMS2270/ILSTS065 markers in the BNP population, where less than 50% (14 of 30) of the tested bison were confirmed to have cattle-like alleles at both loci. This discrepancy is most likely due to recombination between these markers or genotyping error at the ILSTS065 locus, where the absence of a PCR product was interpreted as evidence of a bison-like allele when in fact amplification failure would produce the same result (Halbert et al. 2005). The ILSTS065 locus was coamplified with BMS2270 in the secondary screen in an attempt to eliminate genotyping error.

Both bison-like and domestic cattle–like alleles identified in bison were sequenced and compared with domestic cattle electromorphs for the following microsatellite loci: BM1314, BM4307, BM7145, and BMS2270 (GenBank accession numbers DQ887282–DQ887321). Although the BM4307 197-bp domestic cattle–like allele was detected in 5 different bison populations (Table A1), only bison from FN were sequenced for this microsatellite. Bison from FN were used at least in part in the establishment of each of the other 4 populations (Table 1), and therefore, the FN population is presumed to be the source of the 197-bp allele in the other populations. Similarly, the BM7145 116-bp domestic cattle–like allele was identified in the NBR and NS populations (Table A1) but was sequenced only from NBR bison because the NS population was recently derived in part from NBR bison (Table 1). For each locus, at least one domestic cattle allele was found with 100% identity to the sequence of the bison electromorph presumed to be of domestic cattle origin, thereby supporting the hypothesis that the electromorphs shared between bison and domestic cattle are due to introgression of domestic cattle genomic DNA in bison and not symplesiomorphy or convergence. Further supporting this hypothesis was the identification of single-nucleotide fixed changes between bison alleles and domestic cattle–derived alleles outside of the microsatellite repeat region for BM1314 and BM7145. Analysis of character differences through tree-building algorithms confirmed the common origin of domestic cattle alleles and electromorphs found in bison as well as the separation of these alleles from those of bison origin (Figure 1).

Four bison populations without any evidence of mitochondrial or nuclear introgression were identified in this study: GT, Sully's Hill National Game Preserve (SUH), Wind Cave National Park (WC), and YNP. Previous studies using

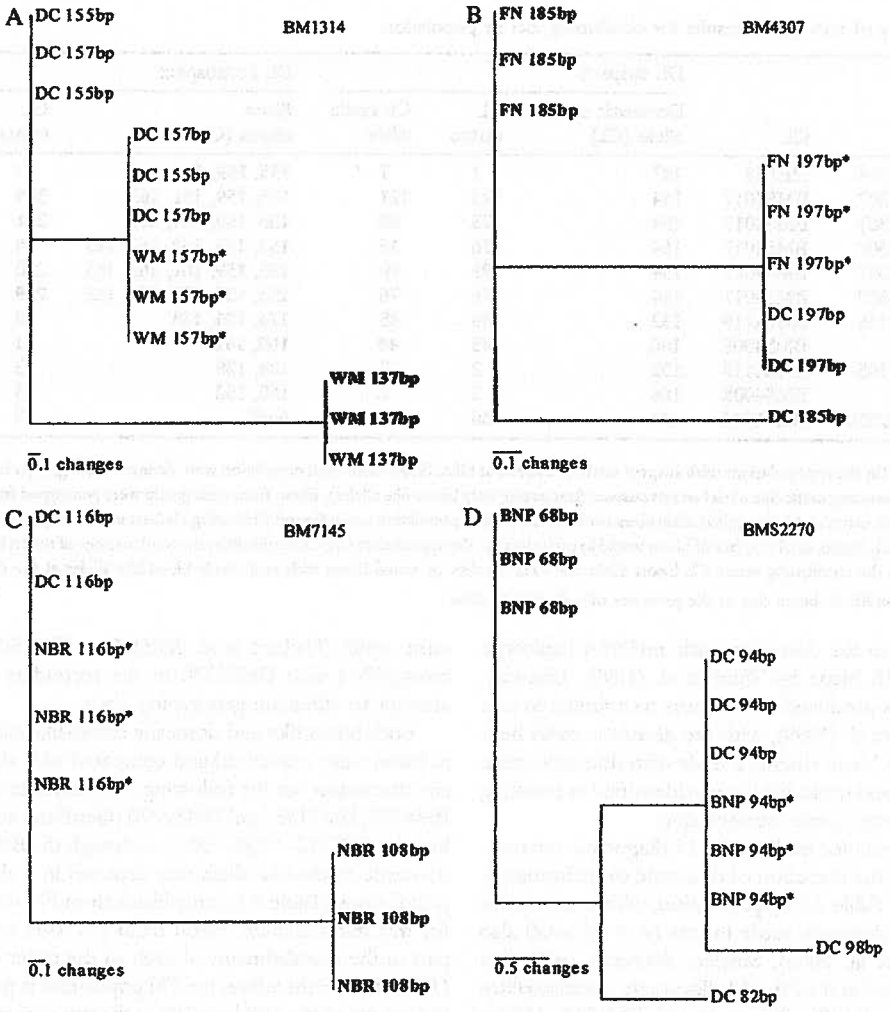

Figure 1. Parsimony analysis depicting relationships among allele sequences for diagnostic markers (A) BM1314, (B) BM4307, (C) BM7145, and (D) BMS2270 using branch-and-bound tree-building algorithms. DC-like alleles found in bison are indicated with an asterisk (*), and true bison alleles are shown in boldface type. Each node represents sequence from a different individual or different allele within an individual. DC, domestic cattle.

smaller sample sizes also failed to identify domestic cattle mitochondrial introgression in YNP and WC bison (Polziehn et al. 1995; Ward et al. 1999) and nuclear introgression in YNP bison (Halbert et al. 2005).

Discussion

In the current study, we identified domestic cattle introgression in some, but not all, tested federal bison populations. A rapid, cost-effective multiplexed PCR assay was developed to facilitate screening of 14 unlinked microsatellite markers on sufficiently large numbers of bison from individual populations. Therefore, we were able to examine populations included in previous studies (Polziehn et al. 1995; Ward et al. 1999; Halbert et al. 2005) in much greater detail for both nuclear and mitochondrial evidence of introgression. Additionally, the prevalence of domestic cattle introgression has been investigated in several federal bison populations for the first time through this study (BNP, GT, NS, SUH, TRN, and TRS). Furthermore, we used both linked confirming microsatellite markers and sequence analysis of diagnostic marker alleles to validate our assay and confirm the origin of domestic cattle–derived alleles in the bison populations examined herein.

Ward et al. (1999) identified domestic cattle mtDNA in 2.7% (3 of 113) of the bison tested bison from NBR, which is comparable to the 1.8% level observed in the current study. A female bison from NBR with domestic cattle mtDNA born in 1984 (see Results) was identified as 1 of the 4 females introduced from Maxwell State Game Refuge (Table 1; Garner L, personal communication). The other 3 females from this introduction were also included in this study; all contained bison mtDNA. The source of domestic cattle mtDNA

introgression in NBR bison is Maxwell State Game Refuge, as corroborated by the following observations: 1) all 9 bison with domestic cattle mtDNA sequenced in this study were identical to those found in NBR bison by Ward et al. (1999), including a single female introduced into the population from Maxwell State Game Refuge, and 2) this haplotype was found to be identical between the 2 populations. Halbert et al. (2005) previously identified domestic cattle–derived alleles of the same size from Maxwell State Game Refuge bison as from NBR bison for the BM7145/INR119/BMS4008 region. In this study, we found approximately 3.8% of the bison examined from NBR contained the same domestic cattle–derived 116-bp allele for BM7145 (Table A1, Figure 1) as that found in Maxwell State Game Refuge (Halbert et al. 2005), which was confirmed with the linked markers INRA119 and BMS4008 (Table 4). However, we did not detect the BM7145 116-bp cattle allele in samples from the 4 females from Maxwell State Game Refuge that were introduced into the NBR population. Furthermore, none of the 11 NBR bison identified as having domestic cattle mtDNA also had the BM1745 116-bp cattle allele. These results indicate 2 independent domestic cattle introgression events in the NBR population, possibly through one or more undocumented bison introductions.

The observation of the same BM4307/BMS4017 domestic cattle alleles in the TR bison populations as found in the FN population is expected based on the history of these populations (Table 1). Two separate regions of domestic cattle introgression were identified in the BNP population (Table A1): one on chromosome 1 (BM4307/BMS4017) and the other on chromosome 24 (BMS2270/ILSTS065). Similarities in allele size and frequency of BM4307 alleles (Table A1) indicate that introductions from FN and TRS were the source of the detected BNP chromosome 1 domestic cattle introgression (Table 1). The BMS2270 94-bp domestic cattle allele, however, is not shared with either TRS or FN and is presumably from the 1984 introduction of bison from Colorado of unknown origin (Table 1). Halbert et al. (2005) also identified domestic cattle introgression in Custer State Park (CSP) bison in the BMS2270/ILSTS065 region. The BMS2270 90-bp allele and ILSTS065 143-bp allele found in CSP (Halbert et al. 2005) are of different sizes, however, from those found in the BNP population (Tables A1 and 4).

The NS bison population shares domestic cattle alleles in the BM4307/BMS4017 region with FN and in the BM7145/INR119/BMS4008 region with NBR, as would be predicted based on the history of this population. The NS population does not share BM1314 domestic cattle alleles with WM, from which 8 bison were used as NS founders (Table 1). Because the frequency of the BM1314 157-bp domestic cattle allele in the WM population is only around 8.9%, it is most likely that this allele was not introduced into the NS population by chance, although drift or unequal contribution of founders might also explain this finding.

Excluding the possibility of recent, undocumented introgression, there are only 2 possible sources of the domestic cattle introgression observed in the BM1314/HEL11 region in the WM bison population: the New York Zoological Park or FN (Table 1). Coder (1975) reported that one of the bulls from the New York Zoological Park was from the Jones herd, where hybridization experiments are known to have occurred. Furthermore, the FN population was supplemented with CSP bison in 1935 and 1937, just before the 1940 transfer of 2 bulls to WM (Table 1). Although domestic cattle introgression was not observed in the BM1314/HEL11 region in the FN population (Table A1), the same alleles found in the WM population (157- and 187-bp, respectively) are found in the CSP population (Halbert et al. 2005). These findings may be the result of genetic drift over the last 60 years to effectively eliminate the introgressed BM1314/HEL11 region from the FN population or the introduction of a single bull directly from CSP through FN to WM that did not produce many, if any, offspring while at FN. The later possibility seems likely based on the timing of the movement of bison among these populations (Table 1).

This study has identified at least 3 federal bison populations with presumed multiple sources of domestic cattle introgression: BNP, NS, and NBR. The importance of utilizing both mtDNA and nuclear loci for the detection of hybridization and introgression was predicted by Rhymer and Simberloff (1996) and is substantiated in this study with results from the NBR population; without both the mtDNA and nuclear loci, the true extent of introgression in this population would have been underestimated. These results also emphasize the importance of the warning given by Simberloff (1996) for extreme caution when purposely mixing individuals from populations, especially when interspecies hybridization is a possible compounding issue. In the case of both the BNP and NBR populations, the observed domestic cattle introgression was in part due to additions made to these populations in the 1980s under the honorable auspices of increasing genetic diversity and limiting inbreeding depression (Berger and Cunningham 1994; Wiseman D, personal communication, respectively).

Hybridization between distinct populations, and in some cases species, is known to increase viability and adaptive response (Spielman and Frankham 1992; Arnold and Hodges 1995), even when the original hybridization is disadvantageous (Lewontin and Birch 1966), as in the case of domestic cattle and bison. Because bison and domestic cattle do not naturally hybridize and there are clear negative fitness consequences in at least the F_1 generation, it seems plausible that the introgression and maintenance of domestic cattle genes into bison germplasm might also be under negative selection. Any potential negative fitness effects are not apparent, however, as the introgressed domestic cattle regions in the populations examined have been maintained for 15–20 generations post-hybridization. However, the location of genes and their respective functions within and near the 14 nuclear regions examined in this study are largely unknown; it is therefore not possible at this point to accurately assess the involvement of natural selection on the maintenance of domestic cattle introgression in these regions.

A total of 3713 bison from 22 US and Canadian populations have been examined for evidence of both domestic cattle mitochondrial and nuclear DNA introgression to date (Ward et al. 1999; Ward 2000; Halbert et al. 2005). Of these,

Table 5. Probability of detection of introgression across a range of individuals sampled (n) from a population using 14 nuclear diagnostic markers (t) across low, conservative ranges of m (level of introgression across population) and p (proportion of domestic cattle genome represented in hybrid founders). Appropriate levels of m were estimated based on detected levels of introgression in extant bison populations by Halbert et al. (2005)

		n (number of individuals sampled per population)											
m	p	25	50	75	100	150	200	250	300	350	400	500	600
0.001	0.250	0.2866	0.4782	0.6092	0.7007	0.8133	0.8746	0.9103	0.9322	0.9463	0.9558	0.9670	0.9730
	0.500	0.2925	0.4950	0.6364	0.7359	0.8570	0.9199	0.9536	0.9722	0.9828	0.9890	0.9951	0.9976
	1.000	0.2954	0.5036	0.6502	0.7536	0.8777	0.9393	0.9699	0.9850	0.9926	0.9963	0.9991	0.9998
0.005	0.250	0.7681	0.9114	0.9521	0.9674	0.9777	0.9807	0.9816	0.9820	0.9821	0.9822	0.9822	0.9822
	0.500	0.8077	0.9541	0.9865	0.9952	0.9990	0.9996	0.9998	0.9999	0.9999	0.9999	0.9999	0.9999
	1.000	0.8270	0.9701	0.9948	0.9991	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
0.010	0.250	0.9128	0.9677	0.9779	0.9807	0.9820	0.9822	0.9822	0.9822	0.9822	0.9822	0.9822	0.9822
	0.500	0.9547	0.9953	0.9990	0.9997	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999
	1.000	0.9703	0.9991	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
0.015	0.250	0.9535	0.9780	0.9814	0.9820	0.9822	0.9822	0.9822	0.9822	0.9822	0.9822	0.9822	0.9822
	0.500	0.9870	0.9990	0.9998	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999
	1.000	0.9956	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

9 populations have been identified with no evidence of domestic cattle introgression (plains bison unless otherwise noted): Elk Island National Park, Canada (wood bison, n = 25); Mackenzie Bison Sanctuary, Canada (wood bison, n = 36); Wood Buffalo National Park, Canada (wood bison, n = 23); Elk Island National Park, Canada (n = 25); GT (n = 39); Henry Mountains, Utah (n = 21); SUH (n = 31); WC (n = 352); and YNP (n = 548, including those from the studies of Ward et al. [1999] and Halbert et al. [2005]). As previously discussed, the ability to detect domestic cattle nuclear introgression in hybrid bison populations is dependent on the proportion of domestic cattle genome represented in the original hybrid founders (p), the average proportion of domestic cattle introgression in each bison (m), the number of individuals sampled (n), and the number of independent, selectively neutral, unlinked diagnostic markers (t) used to detect introgression (Halbert et al. 2005). Given sufficient sample sizes, a high probability of detection using 14 markers is expected even when p and m are low, as indicated in Table 5. In fact, the probability of detection when n = 350 or more, such as for the WC and YNP populations (Table 2), is greater than 94.6% even when assuming a 0.1% level of introgression (m) and only 25% of the domestic cattle genome originally represented in the hybrid founders (p). Although it is not possible to prove unequivocally that domestic cattle introgression does not exist in these populations, our analyses suggest that if introgression does exist, it is most likely at exceedingly low levels ($\ll$0.1% per individual).

Conversely, while domestic cattle nuclear introgression was not detected in the GT and SUH populations, small sample sizes preclude similar confidence in our detection limits. When $n < 50$, as is the case for both the GT and SUH populations in this study, the probability of detection is only around 50% based on a 0.1% level of introgression (Table 5). In fact, we would expect to find domestic cattle introgression in each of these populations based on their histories (Table 1). For example, based on the introduction of FN bison into the SUH population (Table 1), we would expect the BM4307 197-bp domestic cattle allele to have been detected in SUH bison (Table A1). As nearly the entire SUH population was examined in this study (Table 2), the BM4307 197-bp domestic cattle allele most likely either was not introduced with the bison from FN or has been lost from the SUH population due to drift. In either case, it is probable that other regions of domestic cattle introgression, as yet unexamined, exist within the nuclear genome of SUH bison based on the history of this population. Similarly, bison were introduced from TRS into the GT populations in 1964, although the BM4307 197-bp domestic cattle allele identified in the TRS population (derived from FN; Table 1) was not found in the GT population in this study (Table A1). The small sample size from GT (Table 2) may have precluded the detection of domestic cattle introgression at this locus. It is also possible that the contribution of the TRS bison in the 1960s to the GT population was such that the allele in question was not maintained (genetic drift). Further sampling from the GT population, which has a current census size exceeding 600 bison, is necessary to resolve this issue.

The combined results of this study and those of Ward et al. (1999) and Halbert et al. (2005) indicate that relatively few bison populations exist without evidence of domestic cattle introgression, and even fewer have been examined with appropriately large sample sizes to warrant statistical confidence in the detection limits (WC and YNP only). Therefore, further investigation of bison populations without known historic links to populations harboring domestic cattle nuclear introgression, and from which domestic cattle introgression has not been detected, is necessary (e.g., Henry Mountains and several federal Canadian populations, see above). The identification of key sources of germplasm through this study represents a critical step in the long-term conservation of the bison species. Germplasm integrity should be a principle consideration in the establishment of new conservation herds and movement of bison between established herds. For example, to circumvent further degradation of germplasm integrity, bison should not be transferred from hybridized to nonhybridized herds. This study underscores the importance of thorough genetic evaluation of interspecies introgression for wildlife population management and species conservation.

Appendix

Table A1. Allele frequencies for 14 diagnostic microsatellite markers. See Table 1 for sample sizes. Domestic cattle (DC) allele frequencies derived from Halbert et al. (2005). Alien (domestic cattle) alleles detected in bison populations and verified using confirming microsatellites (Table 3) are indicated in bold

	BNP	FN	GT	NBR	NS	SUH	TRN	TRS	WM	WC	YNP	DC
AGLA17												
214												19.53
215	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	
216												1.56
219												78.91
AGLA293												
218	100.00	100.00	100.00	**99.25**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**96.40**	100.00	10.83
220				0.75						3.60		0.83
222												5.83
225												5.00
226												1.67
228												57.50
230												8.33
232												3.33
236												1.67
239												5.00
BM1314												
137	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	90.99	100.00	100.00	
143												0.79
145												0.79
147												7.14
153												9.52
155												42.06
157									**9.01**			29.37
159												4.76
163												2.38
165												1.59
167												1.59
BM4307												
183												1.64
185	59.45	82.09	94.87	89.01	77.78	100.00	73.78	72.30	66.18	89.74	100.00	11.48
187	27.00	4.43	5.13	10.99	8.73		9.97	16.19	33.82	10.26		0.82
189												36.07
191												7.38
197	**13.55**	**13.48**			**13.49**		**16.26**	**11.51**				36.07
199												6.56
BM4513												
132	93.78	94.14	96.15	99.59	90.48	100.00	84.25	100.00	95.35	74.57	82.92	
134	6.22	5.86	3.85	0.41	9.52		15.75		4.65	25.43	17.08	
139												3.13
141												0.78
143												21.09
145												11.72
147												21.09
149												20.31
151												8.59
154												4.69
160												3.91
162												3.13
164												0.78
166												0.78
BM7145												
108	76.33	86.89	98.72	90.88	88.89	100.00	87.01	66.90	100.00	65.90	82.02	
110	23.67	13.11	1.28	5.29	9.52		12.99	33.10		34.10	17.98	
116				**3.83**	**1.59**							87.50
118												12.50

Table continues

Table A1. Continued

	BNP	FN	GT	NBR	NS	SUH	TRN	TRS	WM	WC	YNP	DC
BMS2270												
66	12.20	34.31	30.77	76.75	40.48	50.00	7.44	26.06	64.60	43.60	31.88	
68	84.65	65.69	69.23	23.25	53.97	50.00	92.56	72.25	15.53	37.65	59.21	
70					5.56			1.69	19.88	18.75	8.91	
80												1.59
82												14.29
84												12.70
86												1.59
88												7.94
90												18.25
92												11.90
94	3.15											3.17
96												7.14
98												21.43
BMS4040												
75	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	
85												10.16
87												1.56
97												83.59
98												0.78
99												3.91
CSSM36												
158	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	
162												19.84
167												2.38
169												1.59
171												7.14
173												17.46
175												6.35
177												1.59
179												30.16
181												12.70
185												0.79
CSSM42												
167	69.24	77.99	55.13	62.46	62.26	67.74	54.80	63.57	34.50	67.21	58.51	
169	2.26		2.56	6.44				0.29	22.81	8.01	6.83	
171	28.50	22.01	42.31	31.10	37.74	32.26	45.20	36.14	42.69	24.78	34.65	
173												8.59
175												3.13
177												2.34
179												26.56
181												1.56
193												1.56
205												3.91
207												0.78
209												0.78
211												1.56
213												39.84
217												9.38
RM185												
90												1.61
92	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	0.81
94												7.26
96												10.48
98												4.03
100												12.90
102												36.29
104												9.68
106												16.13
108												0.81

Table continues

Table A1. Continued

	BNP	FN	GT	NBR	NS	SUH	TRN	TRS	WM	WC	YNP	DC
RM500												
123	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	
125												1.67
127												22.50
129												0.83
131												19.17
133												53.33
135												2.50
SPS113												
128				13.08	4.76							
130	85.34	65.44	38.46	56.17	61.11	54.84	53.61	62.54	67.54	54.43	44.61	
132	14.66	34.56	61.54	30.76	34.13	45.16	46.39	37.46	32.46	45.57	55.39	
135												3.17
137												12.70
139												12.70
141												2.38
143												0.79
145												7.94
147												5.56
149												19.84
151												33.33
154												1.59
TGLA227												
73	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	
79												2.50
83												10.00
85												9.17
90												2.50
92												32.50
94												15.83
96												8.33
98												0.83
101												16.67
106												1.67

Acknowledgments

This project was supported by the National Park Service (00CRAG0036), US Geological Survey (00CRAG0020), and Turner Foundation (20011326). We are especially grateful to Drs. Peter Gogan and Ron Heibert and the many federal herd managers and biologists who allowed access to and provided samples from the bison represented in this study. Additionally, we thank Drs. Todd Ward and Robert Schnabel for designing the mtDNA sequencing primers and the initial multiplexed PCRs; Claire Kolenda, Stephanie Krenek, and Natalia Kuhn for technical assistance; and Drs. Joe Templeton and Christopher Seabury for insightful comments and advice.

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Received January 20, 2006
Accepted August 14, 2006

Corresponding Editor: Oliver A. Ryder

Resolving the evolution of extant and extinct ruminants with high-throughput phylogenomics

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Edited by James E. Womack, Texas A&M University, College Station, TX, and approved September 14, 2009 (received for review April 29, 2009)

The Pecorans (higher ruminants) are believed to have rapidly speciated in the Mid-Eocene, resulting in five distinct extant families: Antilocapridae, Giraffidae, Moschidae, Cervidae, and Bovidae. Due to the rapid radiation, the Pecoran phylogeny has proven difficult to resolve, and 11 of the 15 possible rooted phylogenies describing ancestral relationships among the Antilocapridae, Giraffidae, Cervidae, and Bovidae have each been argued as representations of the true phylogeny. Here we demonstrate that a genome-wide single nucleotide polymorphism (SNP) genotyping platform designed for one species can be used to genotype ancient DNA from an extinct species and DNA from species diverged up to 29 million years ago and that the produced genotypes can be used to resolve the phylogeny for this rapidly radiated infraorder. We used a high-throughput assay with 54,693 SNP loci developed for *Bos taurus taurus* to rapidly genotype 678 individuals representing 61 Pecoran species. We produced a highly resolved phylogeny for this diverse group based upon 40,843 genome-wide SNP, which is five times as many informative characters as have previously been analyzed. We also establish a method to amplify and screen genomic information from extinct species, and place *Bison priscus* within the Bovidae. The quality of genotype calls and the placement of samples within a well-supported phylogeny may provide an important test for validating the fidelity and integrity of ancient samples. Finally, we constructed a phylogenomic network to accurately describe the relationships between 48 cattle breeds and facilitate inferences concerning the history of domestication and breed formation.

ancient DNA | Pecorans | domestication

The Pecorans are one of the most diverse groups of mammals, ranging in size from the diminutive duiker (adult weight 9–24 kg, shoulder height 0.45–0.51 m) to the giant giraffe (adult weight 500–1,250 kg, shoulder height 4.5–5.8 m). They are indigenous to all continents except South America and Australia (1) and live in a wide variety of environments. The ruminants are believed to have rapidly radiated in the Mid-Eocene (1), and due to this rapid radiation, the Pecoran phylogeny has proven difficult to resolve, with 11 of the 15 possible rooted phylogenies describing relationships among the Antilocapridae, Giraffidae, Cervidae, and Bovidae having been argued as representations of the true phylogeny (2, 3). A supermatrix analysis of nucleotide sequence data from 16 genes has resolved some of the nodes within the Pecoran "Tree of Life (3)" and has provided the most strongly supported available phylogeny to which we compare the results of our analyses. However, many of the nodes within this phylogeny either have little support or are completely unresolved (e.g., the genus *Caprinae*), and extinct taxa have yet to be phylogenetically placed with confidence (e.g., aurochs). These weakly supported phylogenies have hampered evolutionary studies and conservation efforts for this intriguingly diverse group.

The number and location of prehistoric domestication events for the extinct aurochs (*Bos primigenius*) has also been controversial (4–8), and the ancestry of many of the derived modern breeds of cattle is unknown. Genome-wide single nucleotide polymorphism (SNP) data captured using high-throughput assays provide a method to perform rapid genomic surveys and have recently been used to resolve the history of human populations (9, 10). However, these studies were restricted to a single species, and the remarkable power of these analyses (with >500,000 informative sites) was not fully captured because population relationships depicted using neighbor-joining trees fail to identify multiple ancestral relationships for historically admixed populations. We report an inter-generic, large-scale phylogenomic analysis which applied a genome-wide SNP assay developed for one species to many distantly related species. We also report the application of a genome-wide SNP assay to capture data for ancient DNA samples.

Results

Genotype Fidelity. We have genotyped 16,353 animals representing 61 cattle breeds and 70 species, as divergent from *Bos taurus* as the Savannah elephant (Table S1), with the Illumina BovineSNP50 BeadChip (11, 12) according to Illumina protocols (13). To examine the quality of genotype calls in these outgroup species, we first sequenced the SNP site and flanking regions for

Author contributions: J.E.D. designed research; J.E.D., S.D.M., K.C., A.C., M.C.M., M.M.R., J.W.K., R.D.S., and J.F.T. performed research; M.P.H., K.C., A.C., J.V., C.M.S., A.R.C., G.S.J., R.A.B., O.H., L.S.E., P.W., J.-J.K., K.S.K., T.S.S., C.P.V.T., H.L.N., L.L.C., M.E.B., and G.A.W. contributed new reagents/analytic tools; J.E.D., J.C.P., G.C.C., J.C.M., R.B., R.D.S., and J.F.T. analyzed data; and J.E.D. and J.F.T. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

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This article contains supporting information online at www.pnas.org/cgi/content/full/0904691106/DCSupplemental.

www.pnas.org/cgi/doi/10.1073/pnas.0904691106

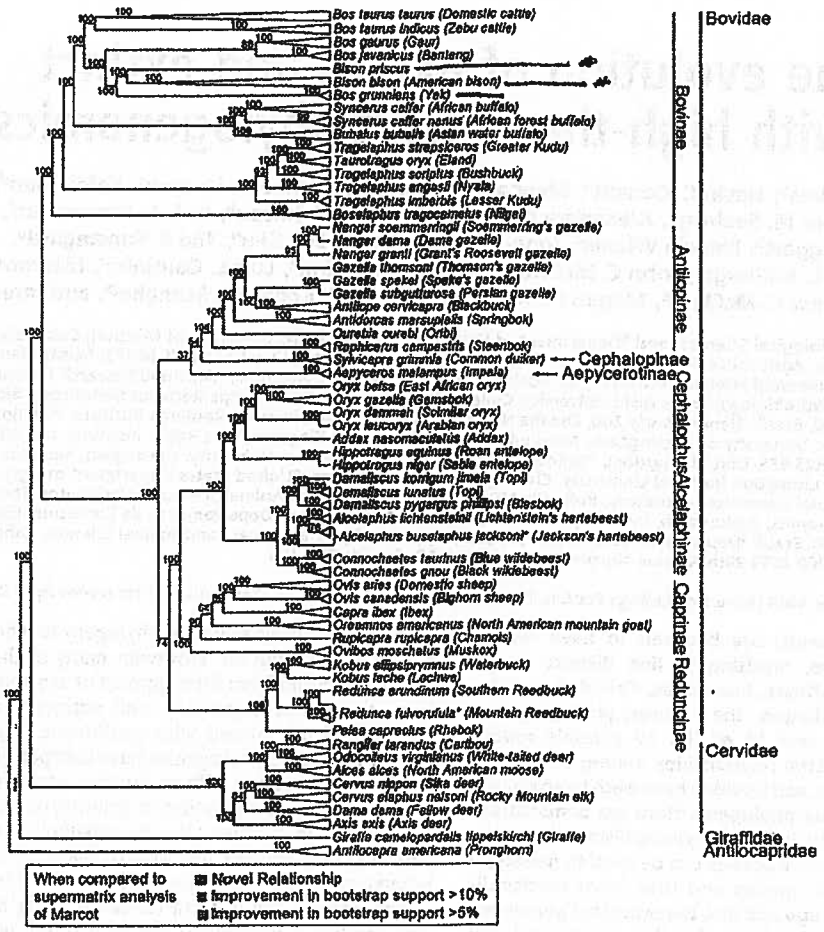

Fig. 1. Strict consensus cladogram (no branch lengths) of 17 most parsimonious trees based on 40,843 SNP genotypes. *, Denotes paraphyletic group.

rs17871403 in 14 species, with pronghorn the most divergent of the sequenced species (Table S2). This SNP was chosen because it has been well characterized in cattle and is a member of a SNP panel that is widely used for parentage analysis (14). Of the genotypes produced by the BovineSNP50 assay (Illumina) for this SNP in these species, 99.13% were concordant with the sequence when we allowed for genotype ambiguity (i.e., *WW* and *SS*) (see *Methods*). One of the six genotyped North American mountain goats and one of the eight genotyped caribou had discordant BovineSNP50 and sequence-based genotype calls (Table S2). This analysis of a single SNP across multiple species suggests a genotyping error rate for BovineSNP50 loci of only 0.87%.

We next aligned all 40,843 SNP probe sequences, which are 50 bases in length, to the international sheep genomics consortium (www.sheephapmap.org) genome assembly (available at https://isgcdata.agresearch.co.nz/ and in an annotated form at http://www.livestockgenomics.csiro.au/sheep/oar1.0.php) and found that only 26,098 (63.9%) could be uniquely aligned, primarily due to the incomplete status of the assembly. Of these SNP, 829 had an unknown base (*N*) identified at the position of the SNP, and for the remaining 25,269 SNPs, there were 308,518 genotypes called in 17 sheep. Genotype calls were in agreement with the genotype predicted from the respective sequence base for 298,311 genotypes (96.7%). There were 1,834 heterozygous genotypes and 8,373 genotypes that were homozygous for an allele not predicted by the sequence assembly. This suggests a BovineSNP50 genotyping error rate of between 2.7 and 3.3% in the outgroup species.

Finally, when minor allele frequencies (MAF) averaged over 40,843 SNPs were plotted against average genotype call rates, samples from outgroup species with the lowest call rates had higher than expected MAF (Fig. S1). This appears to be indicative of DNA quality issues since, for example, DNA for the *Capra ibex* samples was extracted from irradiated blood samples that had been stored under refrigeration for several years. On removing these samples, there was almost no correlation between MAF and call rate (Fig. S1). This indicates that as genetic distance from cattle increases and call rate decreases, spurious heterozygote and alternate homozygote genotype calls rarely arise, indicating support for the quality of these data.

Resolution of the Pecoran Phylogeny. Using genotypes for 40,843 SNPs scored with the BovineSNP50 BeadChip (see *Methods*), we produced a completely bifurcating tree with highly supported nodes for 61 Pecoran species, that contains species that diverged up to 29 million years ago (Fig. 1) (15). There were 39,695 parsimony-informative characters using all 678 animals and, remarkably, 21,019 with cattle excluded. Within the Bovidae, only nine nodes had support <100%. We propose 17 relationships and increase the support for 16 previously proposed nodes within the infraorder, when compared to the supermatrix phylogeny of Marcot (3). A striking observation from the phylogeny is that taxonomic classifications of families and subfamilies

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mirror the topology of the cladogram, since higher taxa form monophyletic groups. This is an improvement over earlier phylogenies, as previously questionable groupings are now shown to be monophyletic.

Ancient DNA Samples. Currently, PCR-based and non-PCR-based multiple strand displacement amplification (MDA) approaches are used to perform whole genome amplification (16, 17). MDA requires high-quality DNA over 2 kb in length and was found to be inefficient for the ancient bison DNA. Consequently, we used a universal linker-based PCR amplification performed with the GenomePlex Whole Genome Amplification kit (Sigma-Aldrich) to amplify the minute amounts of damaged DNA preserved in bone samples from two ancient Russian *Bison priscus* specimens and test whether the Illumina iSelect platform could be used to analyze samples derived from extinct species. The first, sample BS662, was collected from permafrost deposits at Alyoshkina Zaimka, Siberia, and is approximately 20,000 years old (18). The second, ACAD012, was collected from Sur'ya 5 cave in the Ural Mountains and has been accelerator mass spectrometry radiocarbon dated to 34,460 ± 290 years BP. Due to the low amounts of DNA from the ancient specimens and the short DNA fragment lengths produced in the whole genome amplification of degraded ancient samples, the genotype call rates for these samples were much lower than for modern bison (Table S1). However, when these ancient samples were included in the Bovini phylogeny (Fig. 1), BS662 was basal to the modern *Bison bison* clade as expected, but ACAD012 fell within the modern Hereford cattle clade. When we sequenced several overlapping fragments that had been individually amplified from the hypervariable mitochondrial control region of sample ACAD012, we identified variability within the overlapping regions. This is consistent with the sample having been contaminated with modern DNA or being extremely degraded, as also suggested by our genotype data and consequently the sample was removed from the study. A replicate whole genome amplification (library identification KCMU02) was produced from the *B. priscus* sample used to generate BS662, and when this sample was included in the data set, it was sister to BS662, and both remained sister to modern bison within the phylogeny. However, in the preparation of this library, we avoided the initial DNA fragmentation step within the amplification protocol that appeared to greatly improve the quality and quantity of produced genotypes, as KCMU02 produced a higher genotype call rate (54.9 vs. 45.8%) and far lower heterozygosity (11.5 vs. 39.6%) than did BS662 (Table S3). While only 76.1% of the 12,279 genotypes that were called in both samples were identical, 99.7% of the homozygous genotypes, the only genotype class that has the potential to be phylogenetically informative (see *Methods*), were identical between the replicates.

Relationships Among Cattle Breeds. Phylogenetic relationships were also inferred for 48 cattle breeds (n = 372 animals) (Table S1) using parsimony, with most nodes being highly supported (bootstrap values >70%). To accommodate heterozygotes, data were first coded with heterozygotes as polymorphic (noninformative) and then as an independent character state (see *Methods*). When coded as polymorphic, the topology of the cladogram corresponded to the known geographic origins of breeds (Fig. 2*A*). Interestingly, however, when heterozygotes were coded as distinct characters, the topology changed and no longer clearly reflected the biogeography of breed origins (Fig. 2*B*).

To further resolve the issue of breed origins, we constructed phylogenetic networks which can reveal conflicting signals in the data (Fig. 3 and Fig. S2). In Fig. S2, *Bos taurus indicus* and *Bos taurus taurus* are distinct groups with long edges between the subspecies. Within *B. t. taurus*, using the Reynolds et al. (19) distance metric and parsimony cladograms (Fig. 2), African taurine cattle were inferred to be more divergent from European cattle than are the Asian *B. t. taurus* breeds, with 100% bootstrap support in cladograms (Fig. 2 and Figs. S2 and S3). Because SNP were almost exclusively discovered from European *B. t. taurus* samples (12), there is a strong ascertainment bias toward SNP common within European *B. t. taurus* on the BovineSNP50 BeadChip, leading to severe biases in estimates of genetic distance that have prevented us from accurately dating the nodes separating European, African, and Asian cattle (Figs. S3 and S4). Furthermore, the data were recalcitrant to correction for ascertainment (see *Methods*). The network with individuals at node tips (Fig. 3) appears to accurately depict the admixed nature of many populations, for example, the relationship of Belgian Blue to Holsteins and Shorthorns, and Jersey to Iberian and British breeds. The network also reveals pedigree relationships, with sire HO020740 being an interior node to son HO020879.

Discussion

The genotype validation results suggest that BovineSNP50 genotype errors are uncommon, are randomly distributed, and are independent of call rate in the outgroup species. While *Ovis aries* and *B. taurus* are not the most distantly related species surveyed in this study (Fig. 1), their most recent common ancestor was at the base of the Bovidae clade. The use of *O. aries* as a representative for the other species is supported by its 67.2% genotype call rate (Table S1), which was similar to (±7%), or lower than, that for all species and breeds, with the exceptions of Axis deer, Ibex, and Pronghorns, which had call rates <60%.

Despite large amounts of missing data within outgroup species or for the ancient DNA samples, by constructing a larger initial data matrix, which includes more taxa and data than used in previous analyses (20–23), we have produced a highly-resolved phylogeny for a rapidly radiated infraorder, which includes extant and extinct species and in which relationships between and within families have been unresolved. Common ancestry can confound studies of speciation and the evolutionary origins and importance of particular traits; the highly resolved phylogeny presented here can control for this issue by allowing the use of phylogenetically independent contrasts (24). Further, it facilitates informed conservation efforts, as both ancestral relationships and diversity are clearly defined (25), allowing the identification of species and populations within species to target for preservation. With small data sets, the estimated bootstrap support values can be biased due to the presence of a strong correlation between the samples. Large data sets, such as reported here, accurately estimate the support for internal nodes, since nearly independent pseudosamples can be generated for the construction of bootstrap trees.

We demonstrate that reliable genotypes can be produced from ancient DNA samples, but that more work is needed to optimize amplification and genotyping protocols. We suspect that the much higher than expected heterozygosities for these samples are due either to template damage or the nonspecific binding of small, possibly exogenous, DNA fragments to the SNP probes. Despite challenges in library optimization, we placed replicate *B. priscus* samples as sister to modern bison with strong support and have therefore established the feasibility of high-throughput genotyping of ancient samples. Our results also suggest that the fidelity of the produced genotypes may be assessed by their incorporation into a well-resolved phylogeny and that samples producing unreliable genotypes may be identified and removed from further analysis by this process.

Incongruence between the two breed phylogenies occurred as a result of persistent signatures of admixture, which has been well documented in the histories of several breeds. Thus, the conflicting breed phylogenies oversimplify the complex relationships that exist among populations due to geographic isolation, introgression, migration, and admixture. Networks were effec-

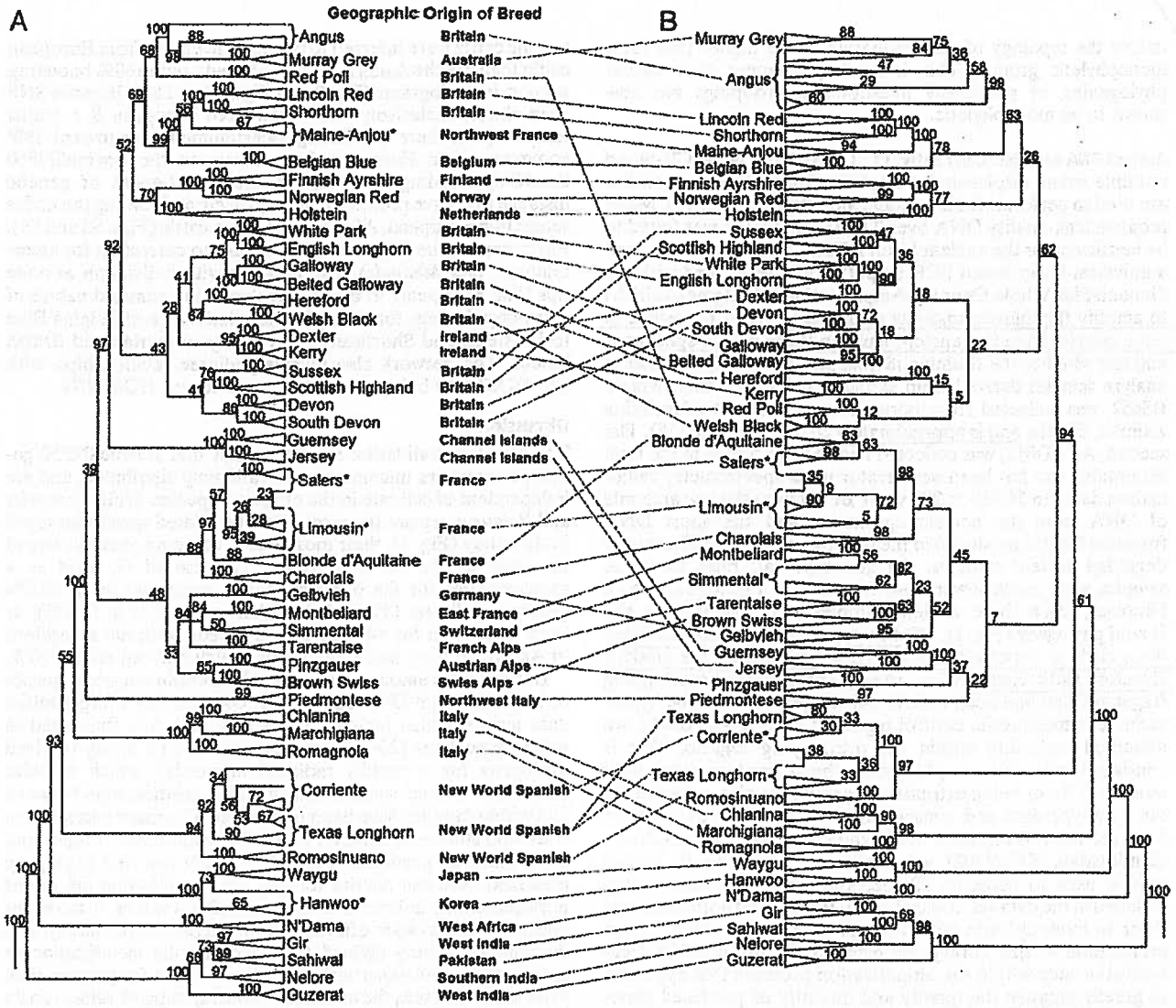

Fig. 2. Consensus of most parsimonious cladograms of 48 cattle breeds. (*A*) Most parsimonious cladogram of 48 cattle breeds with heterozygotes coded as polymorphic. Geographic origins were retrieved from the literature (21). (*B*) Most parsimonious cladogram of 48 cattle breeds with heterozygotes coded as a third and separate character state. Values at nodes are percent bootstrap support from 1,000 pseudoreplicates. Dotted lines connect clades of a breed between the two cladograms. *B. t. indicus* is represented by the Gir, Sahiwal, Nelore, and Guzerat breeds, with all other breeds being *B. t. taurus* (Table S1). *, Denotes paraphyletic group.

tive in revealing both geographic isolation and admixture. There were long branches between *B. t. taurus* and *B. t. indicus*, indicating divergence long before domestication. The networks are also consistent with the biogeography of breeds, with European, East Asian, and African taurine cattle forming separate clusters reflecting a predomestication or early postdomestication divergence for these lineages. The West African *B. t. taurus* N'Dama breed diverges from edges shared with *B. t. indicus* in Fig. 3, and admixture proportions from 0.2–8.6% with African *B. t. indicus* have previously been estimated for N'Dama populations (26). Fig. 3 also reveals the biogeographical history of European cattle, which is based upon migrations out of the Fertile Crescent, with domesticated cattle moved sequentially through Turkey, the Balkans, and Italy (27), then radiating through Central Europe and France, and finally into the British Isles (Figs. 2 and 3 and Figs. S2 and S3). These data also support a second route to the Iberian peninsula by sea from Africa or the Fertile Crescent leading to subsequent admixture with European cattle (4), as the Spanish breeds found in the New World are basal to German and French breeds (Figs. 2 and 3). This pattern of geographic dispersal is interrupted only in a few cases in which breed histories document admixture, such as the Belgian Blue, which was formed between 1840 and 1890 by the crossing of local cattle with Friesian and Shorthorn imported from the Netherlands and England, respectively (28) (Fig. 3). Fig. 3 reveals numerous breed relationships, such as the relationship of the Jersey to both Iberian and British breeds (28), indicating that many exportations and crossbreeding experiments were performed by early pastoralists. Importantly, this figure reveals that the history of breed formation in cattle has been complicated and has involved bottlenecks, evolution in isolation, coancestry, migration, and admixture.

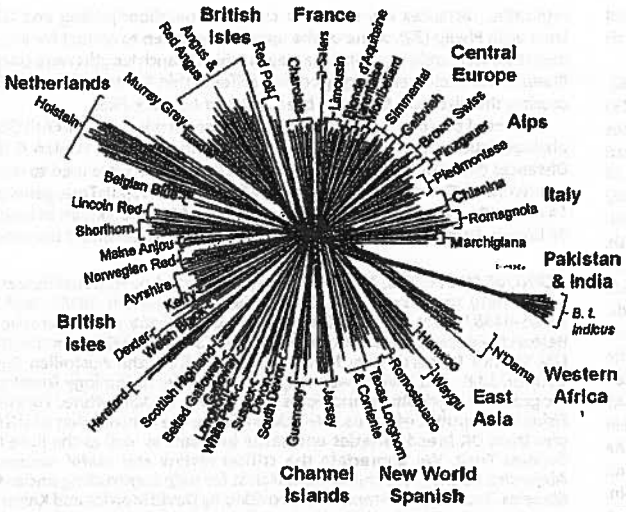

Fig. 3. Phylogenetic network depicting common ancestry for 372 animals representing 48 cattle breeds.

In all analyses, African cattle were the earliest diverged taurine cattle. Consequently, our results now confine the domestication debate to two distinct hypotheses: (*i*) The occurrence of major domestication events in the Fertile Crescent and Indus Valley (7) were followed by minor captures of aurochs in Africa, East Asia, and Europe (4, 6) or (*ii*) three separate domestication events occurred in the Fertile Crescent, Indus Valley, and Africa, with a fourth independent domestication in East Asia less likely (5, 8).

The largest previous supermatrix analysis of artiodactyls included 3,823 parsimony-informative characters and required several years of data collection (3). We produced 21,019 parsimony-informative characters at a rate of 1,152 samples in 6 days for $100 per sample. Where high-density SNP assays are available for sister species, our approach could affordably be applied to the analysis of other orders and families. Such rapid and inexpensive data generation will transform studies of evolution and domestication through the creation of highly resolved phylogenies, including both extant and extinct species. Genome-wide SNP genotyping assays developed for one species can be used for rapid phylogenomic analysis across a broad taxonomic range and are powerful tools for population and evolutionary studies.

Methods

Whole Genome Amplification of Ancient DNA. Ancient DNA was extracted from fossil bison bone specimens using the standard phenol/chloroform/Amicon Ultra-4 method (17). DNA extractions, omniplex library preparations, and PCRs were set-up and performed in a geographically isolated, dedicated ancient DNA facility at the University of Adelaide, Australia. To generate a library of genomic fragments from limited ancient DNA extract, DNA was amplified using the PCR-based GenomePlex Whole Genome Amplification kit (WGA2; Sigma-Aldrich) according to the following protocol: 10 μL DNA were thoroughly mixed with 2 μL library preparation buffer and 1 μL library stabilization solution, and denatured at 95 °C for 2 min. After denaturation, 1 μL library preparation enzyme was added to generate omniplex libraries, followed by a series of incubations at 16 °C for 20 min, 24 °C for 20 min, 37 °C for 20 min, and 75 °C for 5 min in a thermal cycler (Corbett Life Science). The omniplex libraries were next amplified using a limited number of genomic amplification cycles. PCR amplification was conducted in a 75-μL reaction volume containing 14 μL omniplex library, 7.5 μL amplification master mix, 48.5 μL nuclease-free water, and 5 μL WGA DNA polymerase. The PCR amplification conditions were initial denaturation at 95 °C for 3 min, followed by 15 cycles of 94 °C for 15 s and 65 °C for 5 min. GenomePlex-amplified ancient DNA products were finally purified using the GenElute PCR Clean-Up kit (Sigma-Aldrich). Ancient DNA libraries were verified by PCR amplification and sequencing of the hypervariable mtDNA control region before analysis with the BovineSNP50 BeadChip (Illumina). A second amplification, labeled KCMU02, of the sample that produced BS662 was constructed using the same protocol as above, except the genomic fragmentation step within the WGA2 protocol was omitted.

Sample Selection. Table S1 shows the numbers of animals genotyped from each species or cattle breed. In taxa or breeds where <10 animals were genotyped, all animals were sampled. If >10 animals were genotyped, animals with the highest genotype call rates and earliest birth dates were selected. When pedigree information was available, closely related animals were avoided, except in Angus and Holstein where 10 old animals (born in the 1950s, 1960s, and 1970s) and 10 recently born animals (born in the late 1990s and 2000s) were selected. When more than 50 animals within a breed had call rates of at least 98% and no pedigree information was available, 10 animals were sampled at random. Samples belonging to recently formed crossbred breeds were removed from the analysis, as these samples distort parsimony phylogenies. Genotypes for the two ancient bison samples were included despite their much lower genotype call rates, which were expected due to DNA degradation and fragmentation, and the use of whole genome amplification, which affect the fidelity of the Infinium assay. The provenance of all samples included in the analyses is provided in Table S4.

SNP Selection. The BovineSNP50 BeadChip (Illumina) consists of SNP primarily discovered by the sequencing of reduced representation libraries (11), the alignment of random shotgun reads from six cattle breeds to the Hereford assembly, or from the draft assembly of the bovine genome (12). To improve genotype quality for *B. t. indicus* and the outgroup species, we manually adjusted genotype call clusters in Illumina BeadStudio to improve genotype calls. Where pedigree information was available, such as in *O. aries* and *B. bison*, the rate of misinheritances was minimized. A set of 40,843 SNP was selected from the 54,693 loci queried by the assay. Loci selected for analysis were all located on autosomes, had a call rate of at least 80% in 36 (75%) *B. t. taurus* breeds, and were not monomorphic in all breeds. This strategy was effective in selecting informative SNP with few genotype errors (Table S5). Data are available at http://animalsciences.missouri.edu/animalgenomics/publications/php.

Genotype Calls in Outgroup Species. Almost 96% of the beads on the BovineSNP50 BeadChip query Infinium II SNP, in which adenine and thymine share a fluorescent probe and guanine and cytosine share a different fluorescent probe. For samples in which all four bases are present at a single locus, *AA*, *AT*, and *TT* genotypes produce indistinguishable fluorescence intensities, as do *GG*, *GC*, and *CC*. Thus, *A/T* or *C/G* SNP discovered in *B. t. taurus* were limited in the assay design (1.8 and 2.2%, respectively, and use Infinium I chemistry). However, in species diverged from *B. t. taurus* where all four bases could be present, genotypes are *WW* (*W* is the IUPAC code for *A* or *T* bases) for one homozygote class, *SS* (*S* is the IUPAC code for *G* or *C* bases) for the alternate homozygote, and *NN* (ambiguous) for the heterozygote class. This ambiguity is evident when sequences and genotypes for outgroup species were compared (Table S2). The *WW* and *SS* genotypes were identified in BeadStudio as *AA* and *BB* genotype calls.

Phylogenetic Analysis. Most parsimonious trees were inferred from the genotypes using TNT version 1.1 (29). In the analyses involving the outgroup species, phylogenetic signal was obtained only from the homozygous genotypes, and *AA* homozygotes were coded as "0," *BB* homozygotes were coded as "1," heterozygotes were coded as a polymorphic character state (i.e., "[0,1]"), and missing genotypes were coded as "?." However, in the analyses of the cattle breeds, an additional data set was created in which heterozygotes were identified by a unique character state (i.e., *AA* = 0, *AB* = 1, *BB* = 2). A heuristic search was conducting using the search technology in TNT, and the search level was initially set to 20. Specifically, we used the SPR-TBR algorithm followed by random sectorial searches, constrained sectorial searches, exclusive sectorial searches, and 10 rounds of tree-drifting. The complete search was replicated 20 times, with 10 rounds of tree fusing at the conclusion of these 20 replicates. A subset of the samples from the tribe Bovini was independently analyzed along with the ancient bison samples to validate the quality of the data generated from these ancient samples. A data set with 714 samples from all taxon groups was first used to construct the most parsimonious trees. After excluding samples with low quality DNA, low bootstrap support, and/or nonsensical placement in the cladogram (i.e., elephant and horse as sister to *B. taurus*), a final data set with 678 samples was used to construct most parsimonious trees. The cladogram was rooted with *Antiloca-*

pra americana. Using these 678 samples, bootstrap support was calculated using 1,000 pseudoreplicates, and for expediency, the SPR-TBR heuristic search was used.

Allele frequencies were estimated for 40,843 SNP in 22 breeds (Table S6), and these frequencies were used to estimate pairwise Reynolds distances (19) among the breeds (Fig. S3). Several attempts were made to correct estimates of genetic distance for SNP ascertainment bias. First, distances were calculated from haplotype frequencies. Haplotypes were inferred for the autosomes of all genotyped animals in our collection within each breed group (Table S6) using fastPhase (30). From these haplotyped samples, haplotypes were extracted for the study animals for 885 nonoverlapping loci, each comprising six SNP for which the intermarker distance was <50 kb for contiguous SNP. Haplotype frequencies were estimated for each of the 885 loci within each breed group and were used to estimate Reynolds distances between breeds. Next, we formed weighted distances by averaging individual SNP distances weighted according to the frequency of unascertained SNP (31) possessing the MAF observed in each of the two populations. Finally, we also subsampled approximately 3,000 or approximately 8,000 SNP such that the resulting MAF distribution conformed to the unascertained distribution of bovine SNP (31) in Angus or Holstein, respectively. The subsample size was determined by the severity of underrepresentation of SNP within the MAF range 0.005–0.015 and indicates that ascertainment bias was more severe for Angus than for Holstein. Reynolds and Nei genetic distances corrected for sample size (Table S6) were estimated for each subsample and were averaged across 1,000 bootstrap replicates. Distances were used to construct neighbor-joining and UPGMA trees with Phylip (32). None of the approaches taken to correct for ascertainment bias were able to establish a tree in which branch lengths were clock-like. Biases in the allele frequency spectrum differ within *B. t. taurus* breeds (Fig. S4) causing the distances between breeds to not be clock-like.

Figures of phylogenies and cladograms were produced in MrEnt3 (33), and phylogenetic networks were constructed using SplitsTree version 4.10 (34). Distances based upon allele frequencies at 40,843 SNP were used to construct a network of 22 breeds. Due to memory limitations in SplitsTree, genotypes at 14,023 SNP were used to construct a network of 372 individuals belonging to 48 breeds. Default settings in SplitsTree were used to construct the networks.

ACKNOWLEDGMENTS. This project was supported by National Research Initiative (NRI) grant nos. 2006–35616-16697, 2008–35205-18864, and 2008–35205-04687 from the U.S. Department of Agriculture Cooperative State Research, Education, and Extension Service (CSREES), 13321 from the Missouri Life Science Research Board and DP0773602 from the Australian Research Council. J.J.K. and K.W.K. were supported by the Technology Development Program for Agriculture and Forestry, Ministry of Agriculture, Forestry and Fisheries, Republic of Korea. We acknowledge the contribution of DNA samples from UK breed societies and cattle breeders as well as the Rare Breeds Survival Trust. We appreciate the critical review and useful comments of Alejandro Rooney. We thank Oliva Handt for help constructing ancient bison libraries. Technical assistance was provided by David Morrice and Karen Troup (Ark Genomics, The Roslin Institute, Edinburgh, UK). We gratefully acknowledge access to Bovine HapMap Project genotypes.

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Conserv Genet (2010) 11:627–634
DOI 10.1007/s10592-009-9977-y

RESEARCH ARTICLE

Genome variability in European and American bison detected using the BovineSNP50 BeadChip

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Received: 22 May 2009 / Accepted: 14 August 2009 / Published online: 1 September 2009

Abstract The remaining wild populations of bison have all been through severe bottlenecks. The genomic consequences of these bottlenecks present an interesting area to study. Using a very large panel of SNPs developed in *Bos taurus* we have carried out a genome-wide screening on the European bison (*Bison bonasus*; EB) and on two subspecies of American bison: the plains bison (*B. bison bison;* PB) and the wood bison (*B. bison athabascae;* WB). One hundred bison samples were genotyped for 52,978 SNPs along with seven breeds of domestic bovine *Bos taurus*. Only 2,209 of the SNPs were polymorphic in the bison when EB, PB and WB were pooled and only 929 SNPs were polymorphic in EB. Larger numbers of polymorphic SNPs were found in PB (1,403 SNPs) and WB (1,524 SNPs). Also the expected heterozygosity was lower in EB ($H_E = 0.135$) than in WB ($H_E = 0.197$) and PB ($H_E = 0.199$). The polymorphic SNPs were not randomly distributed in the bison, but were aggregated and separated from each other by regions with low haplotype diversity (haplotype blocks). Based on our results we suggest that the utilization of genome-wide screening technologies holds large potential to radically change the breeding practices in captive or managed populations of threatened populations and advocate for developing marker assisted selected strategies in such populations.

Keywords Bottleneck · Founder effect · Marker assisted selection · Genome wide screening · Genetic variation · Inbreeding

Electronic supplementary material The online version of this article (doi:10.1007/s10592-009-9977-y) contains supplementary material, which is available to authorized users.

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Introduction

European bison *Bison bonasus* (EB) were originally distributed throughout vast deciduous forests of Western, Central and Eastern Europe. However, by the beginning of the twentieth century, only two populations remained: one in the Białowieża Forest in Poland (*Bison b. bonasus*) and one in northwest Caucasus (*Bison b. caucasicus*). The last free-living European bison was shot in Poland in 1919, and the last individual in the Caucasus region died before 1927 (Heptner et al. 1966; Pucek et al. 2004). After its extinction in the wild, only 54 (29 males and 25 females) *Bison bonasus* individuals with registered pedigrees survived in

European zoological gardens. The restored population of EB stems from only 7 animals. This breeding line originates from Białowieża Forest and includes pure breed animals of *B. b. bonasus* (Pucek et al. 2004). Consequently, due to the founder effect, the current free-living EB population (about 440 individuals in 2007) is expected to have lower genetic diversity than the historic population.

There are two recognized subspecies of the American bison: the plains bison *Bison bison bison* (PB) and the wood bison *Bison bison athabascae* (WB). About 500 American bison survived the period of intensive subsistence and commercial exploitation during European settlement of the interior of North America in the nineteenth century. Survivors included less than 100 PB (Hedrick 2009) and less than 250 WB (Soper 1941). The population size has increased in the past 100 years and the census population sizes are now estimated to be large for both PB and WB, although most of the bison in North America occur in commercial herds (data after American Bison Society; http://www.americanbisonsocietyonline.org; see also Freese et al. 2007; Hedrick 2009). Coalescent theory supports a demographic model in which the North American bison population rose to a peak around 37,000 years ago and subsequently declined (Shapiro et al. 2004). Thus, although human hunting was responsible for the severe population bottleneck in the North American population during the last 200 years, it seems that the earlier population decline was caused by climatic events which could explain the depletion of the genetic variability of the bison populations prior to the recent bottleneck (Shapiro et al. 2004).

Population genetic variability in extant post-bottleneck populations is poorly known. There are only few data concerning mitochondrial (mtDNA) variation in the EB from the Białowieża Forest (Tiedemann et al. 1998; Burzyńska et al. 1999; Anderung et al. 2006; Wójcik et al. 2009). Molecular markers such as microsatellites and MHC genes have suggested low genetic variability in EB. An investigation of 14 microsatellite loci estimated the mean allele number per locus to be 2.3 (Luenser et al. 2005). Radwan et al. (2007) found only four alleles in the MHC class II DRB3 locus in EB from the Białowieża Forest, one of which (the rarest allele in EB) was identical to a homologous allele in PB.

The main aim of this investigation was to carry out a genome-wide screen of EB from the Białowieża Forest population and of the American bison (subspecies PB and WB) and to determine the consequences of the population bottlenecks that EB, PB and WB have been through. Given the different demographic history of EB, PB and WB we expect to see different levels of genomic variability. Furthermore, the genetic variability was compared among seven breeds of domestic cattle. We genotyped bison and cattle samples for approximately 54,000 single nucleotide polymorphisms (SNPs) across the entire bovine genome. By screening a large number of SNPs, we could considerably reduce the sampling error of the estimated overall genomic variability compared to traditional investigations using a limited number of markers (SNPs or microsatellites). Furthermore, the large amount of data obtained using the BeadChip will facilitate the design of marker assisted selection strategies (MAS) which can be applied for increasing the effective population size (N_e), minimizing genetic drift, increasing generation length and decreasing deleterious effects of inbreeding (Wang and Hill 2000).

Materials and methods

BovineSNP50

The BovineSNP50 BeadChip included more than 54,000 evenly-spaced SNPs. More than half of the SNPs were discovered using the sequencing system Genome Analyzer by Illumina®. The additional SNPs were derived from publicly available sources such as Btau (ftp://ftp.hgsc.bcm.tmc.edu/pub/data/Btaurus/fasta), the bovine reference genome, and the Bovine HapMap Consortium data set (www.bovinehapmap.org). The BeadChip has an average minor allele frequency (MAF) of 0.25 across all loci and has been validated in both dairy and beef cattle. The SNPs are approximately uniformly distributed with an average spacing of 51.5 kb. The number of polymorphic loci varies between breeds. The average call rate over all segregating loci is 99.57% in cattle and was found to be 99.3% in the segregating SNPs when 18 individuals of the species *Bos bison*, *Bos gaurus*, *Bos grunniens*, *Bos javanicus*, *Bubalus depressicornis* and *Syncerus caffer* were analysed. The average call rate refers to the number of useable SNPs. If the genotype of a given animal falls into one of the three genotype clusters that are formed by the SNP it can be "called. The mean MAF of the outgroup consisting of all the species combinedwas found to be 0.05 in 11,206 polymorphic loci (Illumina, Inc. Pub No. 370-2007-029).

Animal samples

A total of 50 EB (from Białowieża Forest, Poland), 25 WB (from the Wood Buffalo National Park, Canada) and 26 PB (from the Elk Island National Park, Canada) specimens were genotyped. The two American bison populations have been identified, together with 7 other herds, as having no evidence of domestic cattle introgression (Halbert and Derr 2007) like the EB population from the Białowieża Forest (Wojcik et al. 2009). The WB population at Wood Buffalo

National Park is one of only two indigenous herds of American bison. It reached a population low of approximately 250 animals in 1895 (Soper 1941). The PB population at Elk Island National Park was founded in the early 1900s by approximately 45 animals (Wilson and Strobeck 1999). Both of these populations have previously been found to be among the most diverse of their subspecies (Wilson and Strobeck 1999). Additionally, 216 *Bos taurus* specimens from seven different dairy and beef cattle breeds (all from Denmark but for some of them with parents from US and Canada) were included in the investigation. These were of the breeds: Red Danish ($n = 32$), Jersey ($n = 31$), Limousine ($n = 30$), Aberdeen Angus ($n = 27$), Hereford ($n = 29$), Holstein ($n = 36$), and Simmental ($n = 31$).

DNA was either isolated from blood using the BioSprint 96 (QIAGEN), or from soft tissue using the DNeasy Blood & Tissue Kit (QIAGEN), or purified from blood and muscle tissue by treatment with proteinase K followed by sodium chloride precipitation (Sambrook et al. 1989).

Infinium II assay protocol

SNPs were genotyped on the BovineSNP50 BeadChip according to the Infinium II Multi-Sample assay protocol provided by Illumina® (Manual Experienced User Card, 11208000 Rev. A., Illumina Inc.). Proprietary reagents were provided by Illumina®. Isothermal amplification was carried out overnight in 96-well plates using 50–200 ng of genomic DNA. The amplification generated a one thousand-fold quantity of DNA.

The amplified products were fragmented by a controlled enzyme process. After alcohol precipitation the DNA samples were re-suspended, denatured and loaded on the BeadChips. The amplified and fragmented DNA samples annealed to locus-specific 50-mers by linking covalently to one of the 54,000 bead types through overnight hybridization. Following hybridization, allelic specificity was conferred by enzymatic base extension. Products were subsequently stained with repeated application of staining and anti-staining reagents. Following staining, the chips were washed and coated. Fluorescence of the beads was detected by the Illumina BeadArray™ Reader.

SNPs were genotyped on the BovineSNP50 BeadChip in two batches with an overlap of six replicate samples (6 individuals were screened twice). These samples came from the EB population where we have extracted DNA from two different tissues (muscle and blood). The first run was conducted on 22 individuals (date: 21 April 2008), of EB, PB and WB including three EB families where the relationships (mother, father and offspring) were known. The second batch was run on all the other bison samples including all samples from the cattle breeds (date: 30 June 2008).

Genotyping of the SNPs

The scanned bead intensities were loaded in the BeadStudio Software for allelic discrimination. All samples, both *Bison* and cattle, were genotyped together and the SNPs were called by applying the Bovine50SNP_A.egt for cluster separation. The samples' reliability was examined by the Call Rate option to remove non-reliable samples. The thresholds were set to 0.96 and 0.98 for *Bison* and cattle, respectively when all polymorphic SNPs were examined in all breeds at the same time. The SNPs were sorted according to the Call Freq option and examined manually if the genotyping frequency was below 0.95. All SNPs segregating in the bison were checked manually to ensure correct calls of clusters and only when the cluster of the bison samples was located within the same range of intensity as the cattle it was accepted. Questionable SNPs were allocated to one of the following categories; unsuccessful reclustering, overlap in clusters, not distinctly separated clusters, low intensity, parent-parent-offspring error or software limitations, and they were excluded from further analyses.

The three EB families utilized for checking of parent-parent–child errors (P–P–c errors) confirmed that the 929 SNPs analysed showed a Mendelian inheritance pattern. No errors were detected among 929 loci suggesting that SNPs can be genotyped reliably and that the true error rate is extremely low. Furthermore, six individuals sampled in the PB population were represented by two different tissue types and run on the system on different dates. These individuals were used to check for replication errors as well as reproducability. All six individuals run twice were 100% identical for all segregating SNPs in the two runs, confirming the reproducibility of the data between runs and tissue types. Besides, the check of mendelian inheritance together with replication of samples confirms, that the finding of the SNPs in bison is correct.

Data analysis

The polymorphic SNPs for bison were plotted by the corresponding known position on the 29 autosomal bovine chromosomes. Given the fact that EB, WB and PB hybridize with each other and that all the bison succesfully hybridize with the different cattle breeds, it is unlikely that the order and distance between the markers is much different between cattle and bison.

The percent of polymorphic loci (P%) for each bison species and for each cattle breed was calculated relative to the total number of loci that were polymorphic when the EB, WB and PB samples were pooled. Expected heterozygosity (H_E) at loci found to be polymorphic in EB, PB and WB and the mean distance (along every chromosome)

between the polymorphic SNPs were estimated. Average observed individual heterozygosity (H_{OI}) was compared between EB, WB and PB by a one-way ANOVA followed by a Tukey's pairwise test.

To test if the distribution of the distances between the polymorphic loci was random, uniform or aggregated along the chromosomes we performed χ^2 analyses for randomness, for EB, PB and WB (Green 1966).

After considering the distribution of the distance between the polymorphic loci the haplotypes were calculated according to the four gamete rule implemented in the Haploview 4.1 software (Barrett et al. 2005). The four gamete rule (under the assumption of random mating) is used to find out if all the four gametes between each pair of SNPs are present. If they are present there is evidence of recombination somewhere between the SNPs. The command line version was used in its default settings (minMAF = 0.001, hwcutoff = 0.001, maxMendel = 1 and minGeno = 0.75) except for the maxDistance parameter which was set to 50,000 kb to ensure correct haplotype blocks partitioning. As input the genotype pedigree file format was used together with the marker info files on each breed and each chromosome separately.

As the number of polymorphic markers in EB, WB and PB was clearly below the amount segregating in the cattle breeds, five decreasing subsets of SNP markers were selected at random from the Jersey breed. The Jersey breed was selected because 1) the Jersey breed segregated in the lowest number of SNPs, 2) the analysis showed that this breed had the lowest amount of blocks with the highest number of SNPs per block and the largest average block size of all cattle breeds, 3) the percentage of SNPs represented in the blocks is highest in the Jersey breed. Based on these finding we predict that the Jersey breed has the lowest genetic diversity of the cattle breeds and hence that it is suitable for comparison to the bison breeds.

Results

A total of 52,978 SNPs were genotyped in the cattle breeds and the European and American bison. There were 42,659 polymorphic SNPs in the seven *Bos taurus* breeds. Only 2,209 of the total amount of SNPs were polymorphic in the bison when EB, PB and WB were pooled. The average call rate for the bison was 97.60% (96.7–98.7%) and 99.57% (98.4–99.9%) for the cattle when all segregating SNPs were considered simultaneously. There were no tendencies for the bison samples to cluster in the interval suggesting the same ability to hybridize to the bovine SNP sequences on the chip in EB, PB and WB. To cluster in the interval, refers to the process of genotyping made by the software, where the clusters of the cattle samples fall all into very narrow intensities (the signal intensity used by the scanner to differentiate the genotypes). The small difference in average call rates (1.97%) confirms the reliability of our comparisons and also reflects differences in the genomic DNA between cattle and bison such as the possibility of

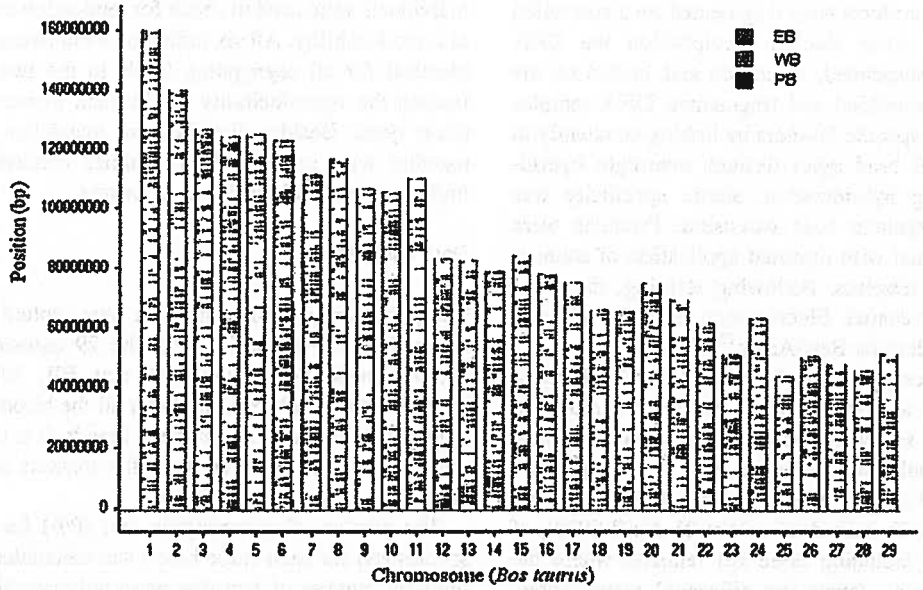

Fig. 1 The distribution of the 829, 1,524 and 1,403 polymorphic SNPs that were mapped to the BTA 4.0 assembly in the European bison (*EB*) (*red*), the wood bison (*WB*) (*green*) and the plains bison (*PB*) (*blue*), respectively. All the polymorphic SNPs are aligned according to their position on the chromosomes

other alleles or deletions of the sequence surrounding the SNPs or in the actual SNP.

A total of 929 SNPs were found to be polymorphic in EB. More polymorphic SNPs were found in WB and PB (1,524 and 1,403 SNPs, respectively, see Fig. 1). Expected heterozygosity was lowest in EB ($H_E = 0.135$) followed by WB ($H_E = 0.197$) and PB ($H_E = 0.199$) (Table 1). In cattle the lowest H_E and P% were found in Jersey ($H_E = 0.278$, P% = 83.30%) and the highest H_E and P% were found in Aberdeen Angus ($H_E = 0.328$, P% = 92.50%) (Table 1).

Differences in H_{OI} among the bison samples were highly significant (one-way ANOVA; $F = 291.2$, $P < 0.001$). Post-hoc tests indicated that H_{OI} of PB and WB did not differ significantly from each other but both have significantly higher H_{OI} variation than EB (Tukey's test $P < 0.001$).

The chromosome alignment in Fig. 1 shows that there are common regions of shared polymorphism in EB, PB and WB. Of the 2,209 SNPs that were found to be polymorphic in at least one of the Bison breeds, 767 were represented in only one of the bison species (EB = 480, PB = 85 and WB = 202). There were clear differences in the distribution of segregating SNPs that were shared among EB, PB and WB (Fig. 2). Very few segregating SNPs were shared only between EB and PB (1.58%) and EB and WB (1.76%) when compared to the overlap between PB and WB (41.1%). Besides, we found that 375 SNPs or 16.98% were represented in all bison species.

The mean distance ± SD. between the polymorphic SNPs in bison was 2,777 kb ± 3,444 kb for EB, 1,870 kb ± 1,965 kb for PB and 1,710 kb ± 2,085 kb for WB. The mean SNP density for EB was 0.36 SNP/Mb, 0.53 SNP/Mb for PB and 0.58 SNP/Mb for WB. The variance of the distribution of the distances between the polymorphic loci in EB, PB and WB along the chromosomes was significantly higher than the mean of the distribution ($P < 0.01$) confirming that the polymorphic loci are aggregated and not randomly or uniformly distributed.

The result of the haplotype block partitioning done on the seven cattle breeds is shown in supplemental Table 1. As pointed out in the methods section the Jersey breed is the less variable of all the breeds. In Table 2 both the Jersey experiment of reducing the amount of SNPs in the dataset as well as the bison haplotype block partitioning is presented. From the data it is evident that the bison are less variable than the Jersey cattle. First of all, the percentages of SNPs represented in the blocks were decreasing rapidly in Jersey with a reduced number of SNPs in the analysis. Secondly, the much longer maximum length of the bison blocks combined with the fact that the blocks contained more SNPs contributed to the lower variability as well. The distribution of SNPs per block in the seven cattle breeds can be seen in supplemental Table 2. Figure 2 illustrates the haplotype block partitioning on BTA 14 in EB, WB and PB and the Jersey dataset containing 1,188 SNPs. From the figure it is evident that EB, WB and PB have greater overlap in the block partitioning than they have with the Jersey breed. Besides, the two American bison subspecies were found to be the most similar. A complete comparison of the bison haplotypes can be seen in supplemental Fig. 1.

Table 1 Overview of the relative degree of polymorphism (P%) and expected heterozygosity (H_E) found in the European bison (EB), plains bison (PB), wood bison (WB) and seven cattle breeds. The percent of polymorphic loci (P%) (5% criterion) for each bison species and for each cattle breed was calculated relative to the total number of loci that were polymorphic when the EB, WB and PB samples were pooled

Sample	(P%)	(H_E)
EB	43.60	0.135
PB	65.20	0.199
WB	70.10	0.197
Red Danish	92.00	0.318
Aberdeen Angus	92.50	0.328
Simmental	90.30	0.31
Limousine	90.80	0.317
Holstein	90.90	0.32
Hereford	90.10	0.313
Jersey	83.30	0.278

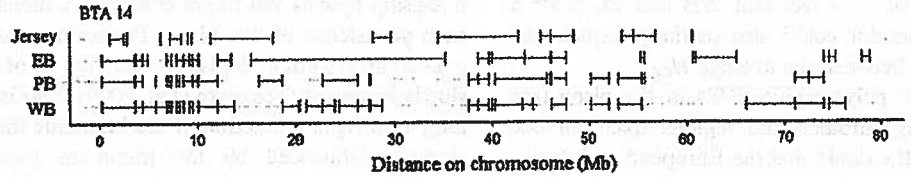

Fig. 2 Haplotype block partitioning of the *Bos taurus* chromosome 14 (BTA 14) in the European bison (*EB*), the plains bison (*PB*) and wood bison (*WB*) together with the haplotype blocks found in Jersey after reduction of the data set. *Hatch* marks of all SNPs in a certain block are connected by a *line*. Each *hatch* represents a SNP

Table 2 Reduction in the amount of single nucleotide polymorphism (SNP) found in Jersey along with haplotype statistics compared to the bison haplotype statistics

Breed	No. of SNPs in total	No. of SNPs in blocks	Percent of SNPs in blocks	No. of blocks	SNP span in blocks	Avg. no. of SNPs per block	Avg. length of blocks (Mb)	Minimum length (Mb)	Maximum length (Mb)
Jersey									
	38,032	29,938	78.7	9,308	2–21	3.2	0.14	0.000001	1.6
	19,016	14,106	74.2	4,731	2–14	3	0.23	0.000001	2.4
	9,508	6,528	68.7	2,347	2–10	2.8	0.41	0.0042	3.5
	4,754	3,077	64.7	1,176	2–8	2.6	0.73	0.0022	5.7
	2,377	1,422	59.8	558	2–7	2.5	1.43	0.024	14.5
	1,188	670	56.4	274	2–6	2.4	2.38	0.039	11.8
Bison									
EB	925	697	75.4	233	2–7	3	3.75	0.024	36.5
PB	1,401	955	68.2	353	2–7	2.7	2.57	0.021	18.9
WB	1,649	1,129	68.5	377	2–12	3	2.39	0.021	17.2

Discussion

We observed a lower level of polymorphism and H_E in EB, compared to WB and PB, which confirms the expectation as the European bison underwent a more extreme bottleneck in population size at the beginning of the twentieth century (seven founders—three females and four males). The EB founder effect was further exacerbated by the skewed genetic contribution of the founder females in the bison population, with one female contributing nearly six times more than the two other females (Wojcik et al. 2009). Furthermore the Y chromosome of all contemporary Lowland line males originates from only one ancestor (Pucek et al. 2004). A certain level of ascertainment bias could have been introduced in this data set, because a bovine DNA chip was used to identify SNPs in two different, although closely related species (genus *Bison*). To alleviate this problem, SNPs were ascertained comparing the EB with the PB and WB data set, and selecting all the segregating sites originating from this comparison. In this way we have minimized the influence of ascertainment bias on the estimated level of genetic variability.

The low variability detected in this study agrees with theoretical expectation for populations which have undergone a severe bottleneck (Nei et al. 1975). Both PB and WB have been through a strong population size reduction (see Soper 1941; Wilson and Strobeck 1999; Freese et al. 2007; Hedrick 2009). The fact that WB and PB share a recent common ancestor could also partially explain the lack of differences between the average H_{Oi}.

Inspection of the polymorphic SNPs in the bison (see Fig. 1) reveals long chromosomal regions fixed for one allele and leaves little doubt that the European and American bison have extremely depauperate genomes. There are several possible reasons for the presence of such haplotype blocks such as genetic hitchhiking, variable mutation rates and recombination, gene-flow, drift and inbreeding (Hayes et al. 2003; Tenesa et al. 2007). It is difficult on basis of the existing data to differentiate between the different possible reasons. Sequencing of the bison genome would help to separate between the reasons for the presence of the haplotype blocks. However, it is likely that many of the shared blocks and of highly polymorphic regions are ancestral as the small N_e of the bison, the relatively low mutation rate of SNPs and the fact that North American bison is a relatively recent evolutionary product, coming into existence about 4,000–5,000 Y.B.P. (Wilson and Strobeck 1999) make it unlikely that the observed polymorphisms are due to mutations that occurred in bison recently. An intensive sequencing of the bison genome, would answer important evolutionary questions in this regard.

No comparison of SNP variation between bison and cattle breeds has been conducted here. This is because an ascertainment bias is introduced when comparing the genetic variability in cattle and bison as the markers on the chip were selected based on polymorphisms in cattle. Hence, the comparisons between bison and cattle should be interpreted with caution. However this random selection of genes would not constitute a problem when comparing genetic variability between cattle breeds.

The low genome-wide level of genetic variability found in EB compared to PB and WB provides the best evidence yet for a low potential to adapt to a variable environment in a flagship species and might constitute a threat to the long term persistence of this bison. Furthermore, despite rapid population growth in the last century the N_e of EB has only slowly increased (Tokarska et al. 2009). This is because the long term N_e is a function of the harmonic mean which is strongly influenced by the minimum population size reached (Lynch and Walsh 1998; Pertoldi et al. 2007).

The domestication of the wild ox or aurochs (*Bos promigenius*), the direct ancestor of the extant cattle

populations, started already 10,000 years B.P. (Bradley et al. 1996). Signs of human manipulation of cattle have also been documented by archeological findings of cattle bones which declined in size with time (Clutton Brook 1999). The different degrees of genetic variability between cattle breeds (Table 1) are due to different N_e and to different demographic history of the breeds. Evidences for selection in cattle on basis of phenotypic traits have been documented in the 18th and 19th centuries (Myrdal 1994). The formal breed definition with herd-books began already 200 years ago. This process has tended to sharpen the differences between breeds and a large amount of genetic variability has been lost during the breeding practices (Lenstra and Bradley 1999).

Genome-wide based breeding schemes designed to preserve rare alleles and minimize inbreeding (by estimating the true relationships between individuals) should be undertaken for the bison and other captive populations. Traditional methods for making breeding decisions to reduce the level of inbreeding (by increasing N_e) utilize only pedigree information, which describes the expected relationship among individuals. With the same pedigree, however, individuals still vary in the realized genetic relationship between them (Nielsen et al. 2007). Therefore, information obtained from genetic markers can be useful in this respect as they will provide the realized genetic relationships. With the information obtained from the BeadChip it will be possible to create a SNP panel on the polymorphisms described here and use them in marker assisted breeding. Information from genome-wide screening also enables detection of genes associated with inbreeding depression and hereditary genetic diseases. SNP-based association mapping has recently been successfully used to identify recessive mutations that cause inherited defects in livestock and dogs (Karlsson et al. 2007; Charlier et al. 2008). Low N_e have increased the rate of inbreeding in many domestic species increasing the expression of recessive deleterious alleles. Thus, detection of recessive deleterious alleles allows rapid control of emerging recessive defects.

Provided the genomic tools are available, similar methods can also be used on small populations held in zoological gardens or under semi-natural conditions. The most effective method to minimize drift and inbreeding is to equalize the contribution of offspring from all potential ancestors. This is generally realized by selecting those individuals for breeding in each generation that have the lowest average coancestry among them (minimum coancestry or equal contribution of parents) (Caballero and Toro 2000). Therefore, the genetic information provided by the SNP chip will allow to start an innovative breeding strategy which uses marker information to select the offspring that have the minimum average probability of identity by descent, which may lead to an increase of both N_e and genetic variability.

SNP arrays are so far only available for a few species. However, our study on bison and an ongoing project using the canine Affymetrix GeneChip to investigate genetic variation in Grey wolves (*Canis lupus*) and domestic dogs (E. Randi, pers. comm.) illustrate that genome-wide scanning is not limited to model organisms, livestock or pet animal species. We expect that these new opportunities will have a huge impact on genetic management of captive populations in the future and potentially revolutionize the field of conservation genetics.

Acknowledgments This study has been partly supported by a Marie Curie Transfer of Knowledge Fellowship BIORESC of European Community's Sixth Framework Program (contract number MTKD-CT-2005-029957), by the Frankfurt Zoological Society—Help for Threatened Wildlife and by the LIFE financial instrument of the European Community (project "Bison Land—European bison conservation in Białowieża Forest", LIFE06 NAT/PL/000105 BISON-LAND). Furthermore, we wish to thank the ConGen program (funded by the European Science Foundation) and the Danish Natural Science Research Council for financial support to CP (grant number: #21-01-0526, #21-03-0125 and #272-08-0567). Finally, we like to thank Kuke Bijlsma and two anonymous reviewers for many helpful suggestions.

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Mitochondrion 11 (2011) 166–175

Contents lists available at ScienceDirect

Mitochondrion

journal homepage: www.elsevier.com/locate/mito

Complete mitochondrial DNA sequence analysis of *Bison bison* and bison–cattle hybrids: Function and phylogeny

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ARTICLE INFO

Article history:
Received 5 March 2010
Received in revised form 5 September 2010
Accepted 14 September 2010
Available online 1 October 2010

Keywords:
Bison
Bos
Phylogenetics
Hybridization
Mitochondria

ABSTRACT

Complete mitochondrial DNA (mtDNA) genomes from 43 bison and bison–cattle hybrids were sequenced and compared with other bovids. Selected animals reflect the historical range and current taxonomic structure of bison. This study identified regions of potential nuclear–mitochondrial incompatibilities in hybrids, provided a complete mtDNA phylogenetic tree for this species, and uncovered evidence of bison population substructure. Seventeen bison haplotypes defined by 66 polymorphic sites were discovered, whereas 728 fixed differences and 86 non-synonymous mutations were identified between bison and bison–cattle hybrid sequences. The potential roles of the mtDNA genome in the function of hybrid animals and bison taxonomy are discussed.

1. Introduction

Due to the vital functions of the mitochondria, it is not surprising that many mitochondrial DNA (mtDNA) mutations have been identified that affect fitness, alter athletic performance, and cause a variety of diseases (Bortot et al., 2009; Florentz et al., 2003; Harrison and Burton, 2006; Tanaka et al., 2010; Wallace, 1994). Most mitochondrial studies have been limited to only a few genes or regions of the mitochondrial genome. However, with the advancement of sequencing technologies, it has become possible to sequence whole mtDNA genomes quickly and accurately. Whole mtDNA genome sequencing has recently revealed important insights into cellular metabolism, mitochondrial gene organization, and genome evolution (Boore et al., 2005). Additionally, whole mtDNA genome sequencing has drastically improved the power and resolution of phylogenetic analysis compared with single gene or single region studies (Santamaria et al., 2007; Simon et al., 2006; Zardoya and Meyer, 1996), allowing for more accurate resolution of taxonomic relationships even at deep levels (Cao et al., 2006; Gissi et al., 2008).

It has been estimated that 1500–2000 nuclear proteins are necessary for the numerous activities of the mitochondria, although only about half of these have been identified to date (Elstner et al., 2008; Prokisch et al., 2006). These nuclear proteins interact with mitochondrial proteins to form co-adapted gene complexes which must remain compatible to ensure mitochondrial function. Reduced fitness levels have been observed among the offspring of both interspecific and intraspecific crosses between populations with different mitochondrial types (Barrientos et al., 1998; Burton et al., 1999; Ellison and Burton, 2008; Liepins and Hennen, 1977; Yamaoka et al., 2000). Additionally, it appears that nuclear–mitochondrial incompatibilities may play an important role in reproductive isolation in fish (Bolnick et al., 2008); however, to date, a mammalian model system to study these effects is lacking.

A well-documented example of interspecific hybridization between American bison (*Bison bison*) and domestic cattle (*Bos taurus*) presents a unique opportunity to examine the effects of mtDNA sequence on nuclear–mitochondrial protein interactions, and possibly on hybrid fitness. The two species, which are members of the same Bovinae subfamily, diverged around 1 million years ago and are largely incompatible: hybridization does not occur naturally and most F_1 offspring are sterile (produced from domestic cow by bison bull crosses) (Goodnight, 1914; Hartl et al., 1988; Jones, 1907; Loftus et al.,

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doi:10.1016/j.mito.2010.09.005

1994). However, a small number of viable bison–cattle hybrids, along with a larger number of purebred bison, were used in the establishment of several bison populations following a dramatic species bottleneck in the late 19th century (Coder, 1975; Garretson, 1938). Unlike first- or second-generation backcrosses, which commonly exhibit morphological characteristics of hybridization, most advanced generation backcrosses are morphologically indistinguishable from purebred bison. As a result, cattle introgression can be found in the nuclear DNA and/or mtDNA genomes of the large majority of bison populations (Halbert and Derr, 2007; Halbert et al., 2005; Vogel et al., 2007; Ward et al., 1999) with around 3.7% of all extant bison harboring cattle mtDNA (Halbert and Derr, unpublished data from more than 10,000 bison in 150 populations).

The relationship between the genera of *Bos* and *Bison* has been a source of debate for decades. In 1758, Linneaus placed bison in the genus *Bos* (*Bos bison*), but bison were subsequently moved to a sister genus (*Bison*) during the 19th century (Wilson and Reeder, 1993). Presently, there are two extant species within the *Bison* genus, the European (*Bison bonasus*) and American bison (*Bison bison*) (McDonald, 1981). The generic distinction of *Bison* has been historically supported by analysis of anatomical distinctiveness (McDonald, 1981; Meagher, 1986; van Zyll de Jong, 1986). However, both species are capable of producing fertile offspring through hybridization with domestic cattle and other members of the genus *Bos* (Boyd, 1908; Goodnight, 1914; Steklenev and Yasinetskaya, 1982) strongly supporting inclusion of *Bison* in the *Bos* genus. Further support for this classification comes from morphological data (Groves, 1981), blood protein analysis (Stormont et al., 1961), phylogenetic analyses of single mitochondrial regions (Burzynska et al., 1999; Janecek et al., 1996; Miyamoto et al., 1989), nuclear ribosomal DNA (Wall et al., 1992), and single nucleotide polymorphism (SNP) analysis (Decker et al., 2009).

Another contentious debate involves the separation of American bison into two subspecies: *B. bison bison* (plains bison) and *B. bison athabascae* (wood bison). The histories of the two lines are similar with extreme population bottlenecks due to environmental and human factors (Isenberg, 2000). In the late 19th century, the wood bison population declined in Canada to an estimated 300 individuals in a single region (now encompassed by Wood Buffalo National Park) (Soper, 1941). Following the enactment of anti-hunting laws, the population increased to 1500–2000 bison. From 1922–1928, approximately 6600 plains bison were imported into the population, leading to a mixture of the two lines (Banfield and Novakowski, 1960; Roe, 1970). Although still somewhat phenotypically distinct, the subspecies designation has been challenged by many (Burton, 1962; Corbet, 1978; van Gelder, 1977; Wilson and Reeder, 1993) and it has been argued that the two are merely ecotypes and not subspecies (Geist, 1991). Furthermore, blood typing, RFLP, and microsatellite DNA analysis have indicated that plains and wood bison are not distinct enough to be considered subspecies (Bork et al., 1991; Peden and Kraay, 1979; Wilson and Strobeck, 1999).

In this study, we sequenced the entire mtDNA genome from 43 American bison and domestic cattle to examine the effects of hybridization between the two species, analyze their phylogenetic relationships, and construct the first whole mtDNA phylogenetic tree of American bison to identify population substructure and subspecific relationships. Bison with native (bison) and non-native (cattle) mtDNA were sequenced to gain insights into differences between the mitochondrial genomes which may contribute to physiological changes in hybrid (bison/cattle) individuals. Synonymous and nonsynonymous differences between bison and hybrid animals, their locations within protein-coding genes, and possible effects on tRNA secondary structure were evaluated. This study is an important step in understanding the mitochondrial sequence diversity found in bison, the role of mitochondrial function in hybridized animals, and the phylogeny of bison in relation to the *Bos* genus.

2. Materials and methods

2.1. Sampling strategy

Whole blood samples were collected between 1997 and 2006. Total genomic DNA was extracted from white blood cells by proteinase K treatment followed by phenol/chloroform extraction (Sambrook et al., 1989). Sample quantity and quality was determined via spectrophotometry (ND-1000; NanoDrop Technologies, Inc., Wilmington, DE). Samples were stored at −80 °C prior to use.

Complete mtDNA sequences were obtained from 43 bison and 3 cattle (Supplemental Materials Table 1) using the following methods described. Each of the bison samples were evaluated for the presence of domestic cattle mtDNA using a PCR-based assay as previously described (Ward et al., 1999). Based on historical records and previous genetic studies, all known extant bison are derived from a handful of foundation herds established in the late 19th and 20th centuries (Coder, 1975; Garretson, 1938; Soper, 1941). To maximize haplotype diversity, efforts were made to include representative haplotypes across 5 of the foundation herds including: Yellowstone National Park (Wyoming, USA), Fort Niobrara National Wildlife Refuge (Nebraska, USA), National Bison Range (Montana, USA), Texas State Bison Herd (Texas, USA), and Elk Island National Park (Alberta, Canada). From these herds, 5 plains bison (*Bison bison bison*) samples and 2 wood bison samples (*Bison bison athabascae*) determined to contain bison mtDNA were selected for sequencing. Additionally, 36 samples from a private bison population recently created from multiple sources were evaluated, including 24 with bison mtDNA and 12 with domestic cattle mtDNA (hereafter referred to as "hybrids") (Supplemental Materials Table 1).

2.2. Sequencing strategy

A rapid method for sequencing both cattle and bison complete mtDNA was developed using the following strategy. Complete mtDNA sequences from human, mouse, and cattle were downloaded from publicly available databases and aligned using Clustal X (Larkin et al., 2007). Primers were designed in highly conserved regions where possible. Pairs of primers were selected across the entire bovine mtDNA genome based on the following specifications: 1) amplified fragment size of 900–1000 base pairs (bp) to allow for direct sequencing using the same (PCR) primers; 2) at least 100 bp of overlap between adjacent pairs to ensure complete sequencing coverage; 3) optimal annealing temperature of 54 °C. These criteria were necessarily adjusted in regions of low conservation. A total of 24 primer pairs were selected (Supplemental Materials Table 2) with an average estimated fragment size of 905 bp (±125.5 bp) and an average overlap of 202 bp (±99.4 bp).

A standard 25 μL PCR mixture was utilized for all primer pairs, including 100 ng template DNA, 1.5 mM $MgCl_2$, 0.025 mM each dNTP, 1× MasterAmp PCR Enhancer with betaine (Epicentre), 0.048 μM each primer, 1× GeneAmp PCR Buffer II and 0.2 μL AmpliTaq® Gold DNA polymerase (Applied Biosystems). Touch-down PCR amplification was performed on a GeneAmp PCR System 9700 (Applied Biosystems) under the following conditions: initial denaturation for 3 min at 96 °C; 3 cycles of 96 °C for 20 s, 57 °C for 30 s, and 72 °C for 1 min with a decrease in annealing temperature of 1 °C during each cycle; 37 cycles of 96 °C for 20 s, 54 °C for 30 s, and 72 °C for 1 min; final extension of 72 °C for 10 min. Resultant amplicons were visualized on 1% agarose gels stained with ethidium bromide and purified with a QIAquick® PCR purification kit following the manufacturer's recommendations (Qiagen).

Bi-directional dye terminator (BigDye® v1.1, Applied Biosystems) sequencing was performed in 10 μL reactions including 90 ng template and 10 pmol primer following the manufacturer's recommendations. Sequence products were purified using sephadex G-50 columns (BioMax) and visualized on a 3130*xl* Genetic Analyzer (Applied Biosystems). Analyzed data was evaluated and assembled into contigs using Sequencher™ 4.9 (Gene Codes Corporation).

2.3. mtDNA genome analysis

The complete mitochondrial sequences of 30 *Bos taurus* (domestic cattle, hereafter "cattle"), 2 *Bos indicus* (zebu), 3 *Bos grunniens* (yak), 1 *Bison bison* (American bison), and 1 *Bubalus bubalis* (water buffalo) were obtained from GenBank (Benson et al., 2005) (Supplemental Materials Table 1). The complete mitochondrial genome sequences were aligned using Clustal X (Larkin et al., 2007) in the MEGA 4.0 phylogenetic package and corrected by hand when necessary. The genomes were parsed into the following data sets for functional and phylogenetic analyses: 1) bison only; 2) bison and hybrids and 3) *Bos*, bison, and hybrids. To investigate the variation among bison mtDNA genomes, an unrooted haplotype network (spring tree) was created using TCS v1.21 (Clement et al., 2000).

2.4. Bison–cattle hybridization analysis parameters

An alignment of bison and hybrid haplotypes was created to assess differences in the two genomes. A previously published cattle mtDNA sequence (AB074966.1) was used to identify putative gene boundaries and overlapping regions. Aligned files were analyzed gene-by-gene in DnaSP v5 (Librado and Rozas, 2009) using the genetic code for mammalian mtDNA to assess the total numbers of polymorphic sites, synonymous changes, and non-synonymous changes. Non-synonymous changes were then coded by amino acid class (non-polar, uncharged polar, positive, negative) to evaluate the number of class-changing mutations between the bison and hybrid groups.

Changes in tRNA structure due to sequence differences between the bison and hybrid groups were evaluated using the online program tRNAscan-SE (Lowe and Eddy, 1997; http://lowelab.ucsc.edu/tRNAscan-SE/). The cove only method (cut-off score = 20 bits) and the mito/chloroplast source definition were used. Locations of changes were recorded in the following categories: D loop/stem, T C loop/stem, central loop, anticodon loop/stem, or acceptor arm stem.

2.5. Phylogenetic analysis

RAxML version 7.0.3 was used to generate unweighted maximum likelihood phylogenetic trees (Stamatakis, 2006). The GTRGAMMA model was utilized, and 1000 replicates were used to generate bootstrap values. To account for different nucleotide substitution rates across the mitochondrial genome, we partitioned the sequence as follows: each of the 13 protein-coding genes and the 2 rDNA genes were treated as independent regions (15 total); the 22 tDNA genes were grouped into one large region; and the D loop was defined as an independent region. For each partition, individual alpha-shape

Table 1
Annotation and gene organization of the *Bison bison* mitochondrial genome. Order, gene, strand (heavy or light), starting and ending nucleotide position, first and last ten bases, and intergenic/overlapping nucleotides ("–" indicates overlapping regions) are based on whole mtDNA genome alignment of all bison haplotypes and comparison with published cattle sequences.

Order	Gene	Strand	Start	End	Length (bp)	First ten bases	Last ten bases	Intergenic nucleotides
	D loop		1	364	362–364	ACTAATGGCT	CCCCCCCCCC	0
1	tRNA-Phe	Heavy	365	431	67	GTTGATGTAG	TCCATAAACA	0
2	12s-rRNA	Heavy	432	1387	956	CATAGGTTTG	TTGGATAAAT	0
3	tRNA-Val	Heavy	1388	1454	67	CAAGATATAG	AATATCTTGA	0
4	16S rRNA	Heavy	1455	3025	1570–1571[d]	ACTAAATCTA	ACAGGGCTTA	0
5	tRNA-Leu	Heavy	3026	3100	75	GTTAAGGTGG	CTCCTTAACA	2 (AA)
6	ND1	Heavy	3103	4058	956	ATGTTCATAA	CGCAAACATA[a]	0
7	tRNA-Ile	Heavy	4059	4127	69	AGAAATATGT	CTTATTTCTA	−3 (CTA)
8	tRNA-Gln	Light	4125	4196	72	CTAGAACTAT	CCAAATTCTA	2 (TT)
9	tRNA-Met	Heavy	4199	4267	69	AGTAAGGTCA	TCCCGTACTA	0
10	ND2	Heavy	4268	5309	1042	ATAAATCCAA	GTATTAGAAT[a]	0
11	tRNA-Trp	Heavy	5310	5376	67	AGGAATTTAG	TTAATTCCTC	1 (C)
12	tRNA-Ala	Light	5378	5446	69	TAAGGATTGC	CTAAATCCTC	1 (A)
13	tRNA-Asn	Light	5448	5521	74[b]	CTAGACTGGT	CTTCAATCTA	0
	L-strand ori. of rep.	Light	5522	5553	32	CTTCTCCCGC	AAGGCGGGAG	0
14	tRNA-Cys	Light	5554	5620	67	AAGCCCCGGC	CCACAGGGCT	0
15	tRNA-Tyr	Light	5621	5688	68	TGGTAAAAAG	CCATTTTACC	1 (C)
16	COI	Heavy	5690	7234	1545	ATGTTCATTA	CCTAAAATAA	−3 (TAA)
17	tRNA-Ser	Light	7232	7304	71–73[d]	TAAGAAAGGA	TCTCTCTCAA	4 (TAAA)
18	tRNA-Asp	Heavy	7309	7377	69	CGAAGTGTTA	GTACACCTCA	1 (T)
19	COII	Heavy	7379	8062	684	ATGGCATACC	AATATTATAA	3 (AAT)
20	tRNA-Lys	Heavy	8066	8132	67	CACCAAGAAG	TCCTTGGTGA	1 (C)
21	ATP8	Heavy	8134	8334	201	ATGCCACAAC	ACCCCTATAA	−40
22	ATP6	Heavy	8295	8975	681	ATGAACGAAA	CAACACATAA	−1 (A)
23	COIII	Heavy	8975	9755	781	ATGACACACC	TGATGAGGCT[a]	3 (CCT)
24	tRNA-Gly	Heavy	9759	9827	69	ATTCTTTTAG	AAAAAGAATA	0
25	ND3	Heavy	9828	10,174	346	ATAAATCTAA	GAACCGAATA[a]	0
26	tRNA-Arg	Heavy	10,175	10,243	69	TGGTACTTAG	TAATTACCAA	0
27	ND4 (L)	Light	10,244	10,540	297	ATGTCTATAG	CCAATGCTAA	−7
28	ND4	Heavy	10,534	11,911	1378	ATGCTAAAAT	CCTCTATACT[a]	0
29	tRNA-His	Heavy	11,912	11,981	70	GTAAATATAG	CTTATTTACC	0
30	tRNA-Ser2	Heavy	11,982	12,041	60	GAAAAAGTAT	GGCTTTTTCG	1 (A)
31	tRNA-Leu2	Heavy	12,043	12,112	70[c]	ACTTTTAAAG	AATAAAAGTA	0
32	ND5	Heavy	12,113	13,933	1821	ATAAACATAT	CCACGAGTAA	−17
33	ND6	Light	13,917	14,444	528	TTAATTTCCA	ATAGTATCAT	0
34	tRNA-Glu	Light	14,445	14,513	69	TATTCTTACA	CTACAAGAAC	4 (ACTA)
35	CYTB	Heavy	14,518	15,657	1140	ATGACTARCC	AAAATGAAGA	4 (CAGG)
36	tRNA-Thr	Heavy	15,662	15,730	69	TCTTTGTAGT	CCCTAAGACT	−1 (T)
37	tRNA-Pro	Light	15,730	15,795	66	TCAAGGAAGA	CTATTCCCTG	0
	D loop		15,796	16,325	528–530	AACCCTATTA	ATCTCGATGG	0

[a] TAA stop codon completed by addition of 3' adenine residues to mRNA.
[b] Region has a fixed insertion in bison as compared to cattle.
[c] Region has a fixed deletion in bison as compared to cattle.
[d] Indel present within gene.

parameters, GTR-rates, and empirical base frequencies were estimated and optimized creating individual nucleotide substitution models. The coordinates for all regions were determined from the multiple alignment and previously published coordinates. The *B. bubalus* sequence was used as the outgroup for the *Bos/Bison* phylogenetic tree, and the yak sequences were used as the outgroup for the bison phylogenetic tree.

3. Results

3.1. Description of bison mitochondrial genome

Complete mtDNA genomes from the 43 bison and 3 cattle sequenced in this study were deposited in the GenBank database (accession numbers GU946976–GU947021). The complete *Bison bison*

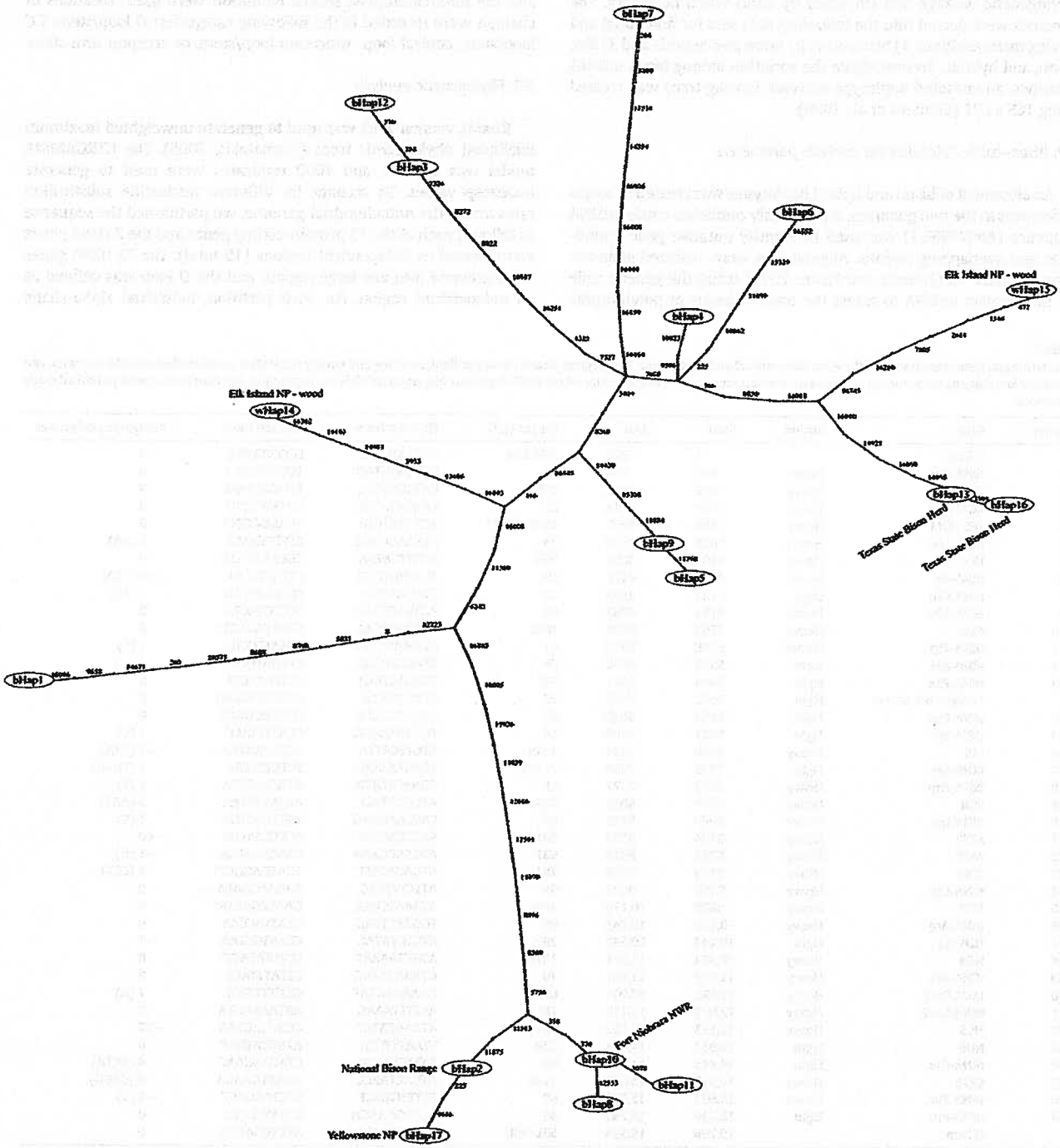

Fig. 1. Bison haplotype network. A spring tree network of 16 bison mtDNA genome haplotypes identified in this study and 1 previously published bison mtDNA genome (bHap1; Achilli et al., 2008) was created in TCS v1.21. Polymorphism locations are indicated by numbers between nodes (see Table 1 for gene location information). Haplotypes representing individuals from historically significant bison herds are indicated alongside the appropriate node (NP, National Park; NWR, National Wildlife Refuge).

mitochondrial genome is 16318–16323 bp long (Supplemental Materials Table 1) consisting of 13 protein-coding genes, 22 tDNA genes, 2 rDNA genes, and the D-Loop region. The gene order, length, and gene coordinates for the mtDNA genome are presented in Table 1. The gene order is conserved between bison and cattle. Including both SNPs and insertion–deletion events (indels), there are 989–995 differences between the bison haplotypes and the cattle genomic reference sequence. The length of the D-loop region varies by 4 bp across bison due to indels. Indels are also present in two genes within bison: 16s rRNA has a 1 bp indel at position 1880 and tRNA serine has a 2-bp variable indel at position 7301–7302. Furthermore, the stop codon for five protein-coding genes is completed by the addition of 3′ adenine residues to the mRNA (Table 1) as previously reported in cattle and other species (Achilli et al., 2008; Boore et al., 2005). There are also several regions in the bison genome where intergenic nucleotides exist or where two genes overlap (Table 1). Whether these regions represent a biological phenomenon or annotation errors is unknown; however, intergenic nucleotides have been reported in other species (Achilli et al., 2008; Boore et al., 2005).

In total, the 17 bison haplotypes included 66 polymorphic sites, 34 singleton variable sites (SNP found in only one animal), and a pair-wise average of 15.3 differences between any two sequences. For comparison, the 39 cattle haplotypes included 426 polymorphic sites, 261 singleton sites, and a pair-wise average of 46.2 differences. These results lead to a 3-fold lower nucleotide diversity (π) value for the analyzed bison haplotypes ($\pi = 0.00094$) than the cattle haplotypes ($\pi = 0.00283$). To illustrate the total number of differences among bison haplotypes, an unrooted haplotype network was created to map the number and position of polymorphisms (Fig. 1).

3.2. Bison–cattle hybridization analysis

Sequence analysis of the 13 protein-coding genes in bison compared to their homologs in hybrid animals revealed a large amount of sequence variation (Table 2). A total of 777 polymorphic sites were identified between bison and hybrid sequences with 728 fixed differences between the two groups. Of the fixed mutations, 642 synonymous and 86 non-synonymous mutations were identified.

To further assess the potential effects of the non-synonymous mutations on protein structure, we identified a total of 40 mutations predicted to cause an amino acid class change. The remaining 46 non-synonymous mutations result in amino acid substitutions within the same class (e.g., non-polar to non-polar).

We also examined the effects of SNPs and indels on tRNA structures between bison and hybrid haplotypes (Table 3). Our analyses indicate that 16 of the 22 tRNA genes annotated have a predicted sequence change in the D loop, T C loop, central loop, or one of the stems of the tRNA and that 9 of these have a sequence change at more than one of the stems and/or loops. A bulge or mis-pairing of nucleotides due to a 1 bp deletion in the hybrid haplotypes was predicted for tRNA-Asn (Fig. 2) and tRNA-Leu. All anticodon sequences were conserved between the bison and hybrid groups.

3.3. Phylogenetic analysis

Phylogenetic analysis of the *Bison* and *Bos* lineages is presented in Fig. 3 (see Supplemental Materials Table 3 for complete SNP table). Two major clades were identified in this analysis: the indicus/taurus clade including *Bos taurus*, *Bos indicus*, and hybrids, and the bison/yak clade including bison (plains and wood) and *Bos grunniens*. Within the indicus/taurus clade, *B. indicus* and *B. taurus* form into separate sub-clades (Fig. 3, I and II, respectively). According to our analysis, cHap56, which was only defined as "'Beef Cattle', Korea" by Achilli et al. (2008), is in the *B. indicus* lineage rather than the *B. taurus* lineage.

Within the taurus sub-clade, strong bootstrap values also support the grouping of cHap51–cHap54 (Chianina, Romagnola, Cinisara, and Agerolese cattle breeds) and cHap49, 50, and 55 (Romagnola, Chianina and Italian Red Pied cattle breeds) (Fig. 3, V and IV respectively). Another statistically significant branching event forms two sub-clades: one including three Angus haplotypes (cHap33, cHap35, and cHap19); and one including the Japanese Black, Angus, and all of the hybrid haplotypes (Fig. 3, III and II respectively). All the hybrid haplotypes group into one sub-clade although this branching pattern is not statistically significant.

We also examined the phylogenetic differences between the mtDNA genome sequences of Bull 86 and its clone, Bull 86^2 (Westhusin et al., 2007). Bull 86^2 harbors a mtDNA haplotype from the recipient egg cell used in the cloning procedure and therefore does not share the same mtDNA haplotype as the original Bull 86. In fact, the two genomes (cHap18 and cHap19 from Bull 86 and Bull 86^2, respectively) differ by 18 SNPs and 1 indel, and fall into two distinct cattle sub-clades (II and III, respectively). To our knowledge, this is the first mtDNA genome sequence and phylogenetic comparison of an animal and its clone. This type of information may prove valuable in understanding phenotypic variability among clones.

The bison/yak clade consists of two sub-clades divided by species: *Bison bison* (plains and wood bison; Fig. 3, VI) and *Bos grunniens* (yak; Fig. 3, VII). The large amount of analyzed sequence and high bootstrap values support the inclusion of the *Bison* genus within the *Bos* genus, with yak being more closely related to bison than to *Bos indicus* or *Bos taurus*.

A more detailed analysis of plains and wood bison lineages reveals significant population substructure with highly significant bootstrap values (Fig. 4; see Supplemental Materials Table 4 for complete SNP table). Using yak as an outgroup, we analyzed the substructure of the representative bison group. A previously published sequence

Table 2
Analysis of protein-coding genes. Sequence variations were recorded between bison and bison–cattle hybrid haplotypes.

Gene name	Abbreviation	Fixed synonymous	Fixed non-synonymous	Fixed total	Amino acid class changes	Total polymorphic sites
ATPase 6	ATP6	28	7	35	3	39
ATPase 8	ATP8	9	4	13	3	15
Cytochrome oxidase I	COI	74	1	75	0	78
Cytochrome oxidase II	COII	43	3	46	2	47
Cytochrome oxidase III	COIII	40	6	46	4	51
Cytochrome *b*	CYTB	67	11	78	4	85
NADH dehydrogenase 1	ND1	56	5	61	3	63
NADH dehydrogenase 2	ND2	64	6	70	3	74
NADH dehydrogenase 3	ND3	17	1	18	1	18
NADH dehydrogenase 4	ND4	85	10	95	3	104
NADH dehydrogenase 4L	ND4(L)	14	2	16	2	17
NADH dehydrogenase 5	ND5	114	25	139	11	147
NADH dehydrogenase 6	ND6(L)	31	5	36	1	39
	Total	642	86	728	40	777

Table 3
Predicted changes in tRNA structure between bison and bison–cattle hybrids. Gray-shaded squares indicate the predicted position of SNPs in the tRNA structure; black square indicates SNPs in both loop and stem structures.

Order from Table 1	Gene	Fixed differences[a]	Polymorphic sites[b]	D loop/stem	TψC loop/Stem	Central loop	Anticodon loop/stem	Acceptor arm stem
1	tRNA-Phe	0	0					
3	tRNA-Val	0	0					
5	tRNA-Leu	1	1					
7	tRNA-Ile	2	0					
8	tRNA-Gln	2	0					
9	tRNA-Met	2	0					
11	tRNA-Trp	0	0					
12	tRNA-Ala	0	1					
13	tRNA-Asn	6	0					
14	tRNA-Cys	1	0					
15	tRNA-Tyr	1	0					
17	tRNA-Ser	0	3					
18	tRNA-Asp	4	0					
20	tRNA-Lys	2	0					
24	tRNA-Gly	3	0					
26	tRNA-Arg	1	0					
29	tRNA-His	0	0					
30	tRNA-Ser2	4	0					
31	tRNA-Leu2	3	0					
34	tRNA-Glu	1	0					
36	tRNA-Thr	3	0					
37	tRNA-Pro	2	0					

[a]Indicates number of fixed differences between bison and hybrid groups.
[b]Indicates number of polymorphic sites within bison.

roots the bison clade (Achilli et al., 2008) and is divergent from the other bison haplotypes analyzed in this study (Fig. 4, III). Unfortunately, this sequence was obtained from a bison at the Antwerp Zoo in Belgium that was originally procured through the University of Utrecht with no data indicating ancestral geographical origins (Antonio Torroni, personal communication). A major sub-clade (Fig. 4, I) is formed from haplotypes from Fort Niobrara National Wildlife Refuge (bHap10), Yellowstone National Park (bHap17), the National Bison Range (bHap2), and the private bison herd. Another major sub-clade (Fig. 4, II) includes sequences from the Texas State

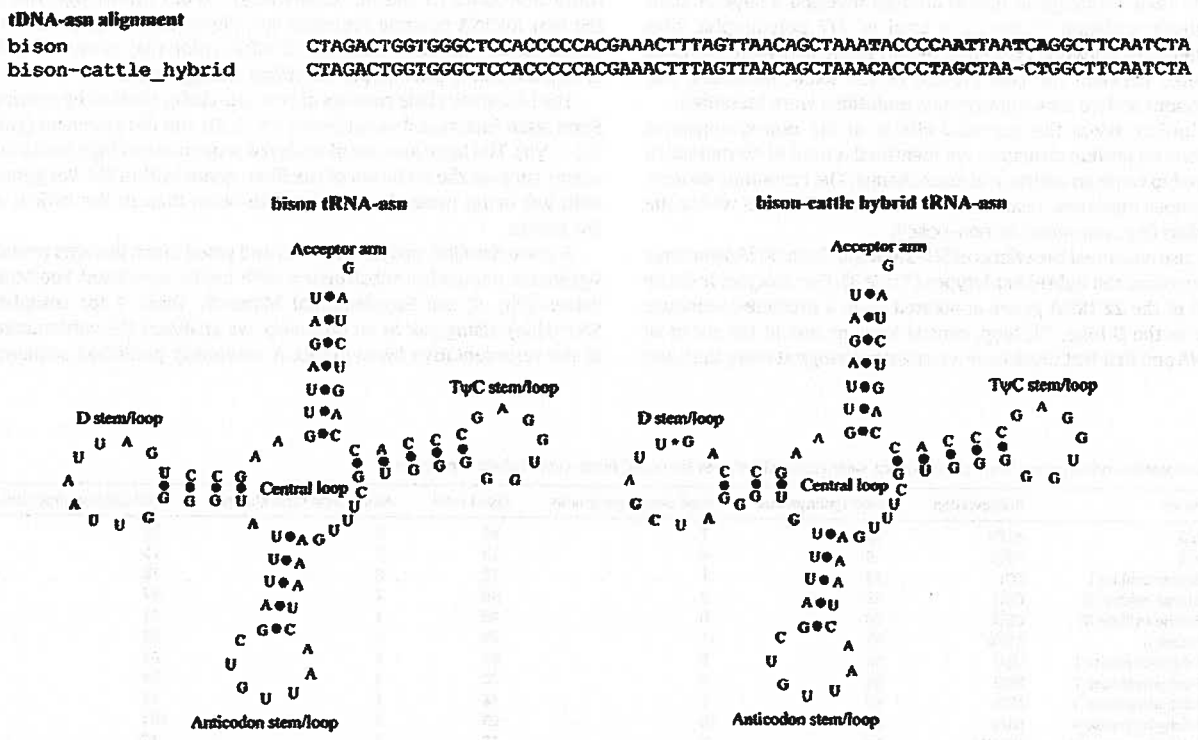

Fig. 2. DNA alignment and predicted tRNA secondary structures of bison and bison–cattle hybrid haplotypes for tRNA-asparagine (asn). The DNA alignment was created in Clustal X (Larkin et al., 2007) and the predicted tRNA secondary structures were created using tRNAscan-SE (Lowe and Eddy, 1997). Differences between the bison and bison–cattle hybrid haplotypes are indicated in bold typeface. An asterisk (*) in the bison–cattle hybrid tRNA structure indicates the location of the indel (missing A nucleotide).

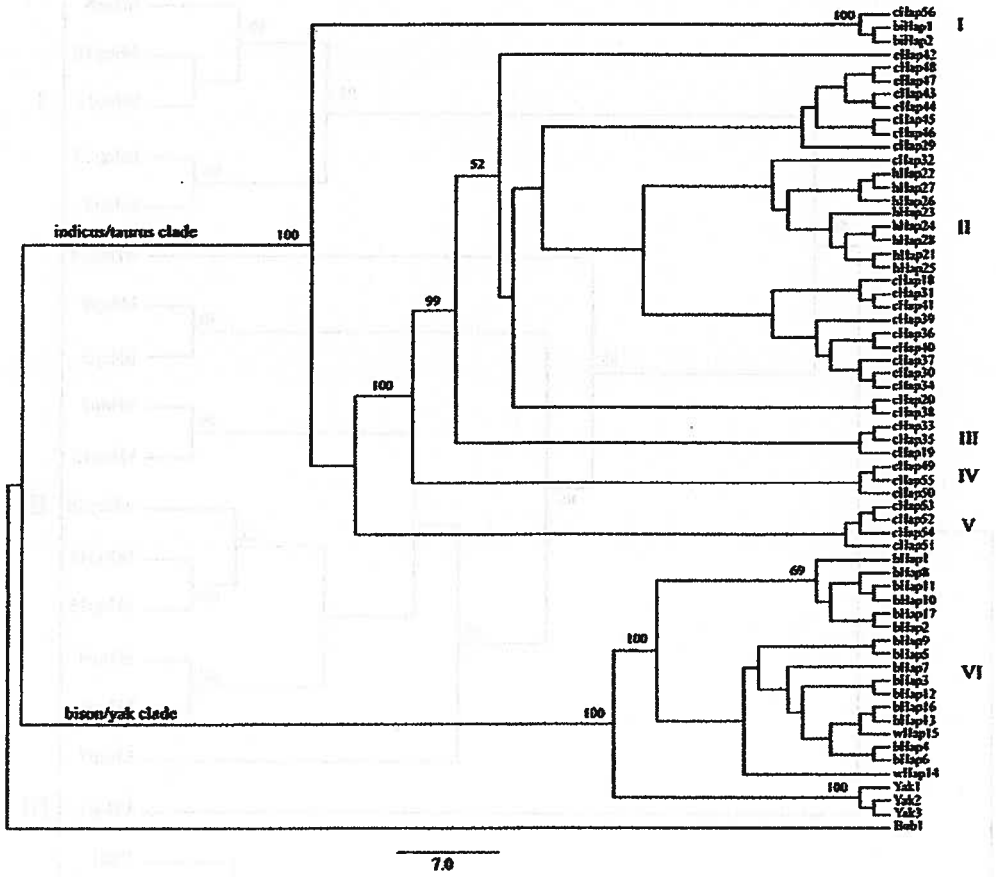

Fig. 3. Maximum likelihood phylogenetic tree of complete mtDNA haplotypes of plains bison *B. bison bison* (indicated with a "b" before haplotype number), wood bison, *B. bison athabascae* (indicated with a "w"), *Bos taurus* (indicated with a "c"), *Bos indicus* (indicated with a "bi"), Hybrid *Bos/Bison* (indicated with a "h"), *Bos grunniens* (Yak 1–3), and *Bubalus bubalis* (Bub1). Clades: I — *Bos indicus*; II — *Bos taurus* and hybrids; III — *Bos taurus* (Angus); IV — *Bos taurus* (Romagnola, Chianina, and Italian Pied); V — *Bos taurus* (Chianina, Romagnola, Cinisara, and Agerolese); VI — bison; and VII — yak. Bootstrap values were determined using 1000 replicates.

Bison Herd (bHap13 and bHap16), the private bison herd, and two wood bison from Elk Island National Park (wHap14 and wHap15), which form unique haplotypes but do not group together.

4. Discussion

Although the bison–cattle hybrids found in nearly all public and private herds are thought to have normal fertility (Halbert and Derr, 2007; Halbert et al., 2005), there are a large number of differences between the mtDNA genomes of the two species (*Bison bison* and *Bos taurus*). By comparing sequences from bison and hybrid animals, we identified at least one non-synonymous mutation in each of the 13 protein-coding genes, with NADH5, cytochrome *b*, and NADH4 harboring the largest number of mutations (25, 11, and 10, respectively; Table 2). In fact, the seven subunits of mitochondrial NADH dehydrogenase, which interact to form one large protein complex, have a total of 54 non-synonymous mutations and 24 amino acid class changes among all 7 subunits. Furthermore, differences between bison and hybrids were identified for 16 of the 22 tRNAs, which may affect tRNA secondary structure and function.

The critical nature of the mitochondria in cellular function and necessary interaction of multiple protein complexes for proper mitochondrial function suggest that the additive effects of such large numbers of non-synonymous mutations will likely affect mitochondrial function and the overall fitness of the organism. It has been demonstrated in species ranging from *Mus musculus* to *Caenorhabditis elegans* that mutations in single protein-coding genes that cause severe mitochondrial disease significantly decrease the fitness of offspring and are often eliminated in the germline. However, less severe mitochondrial gene mutations can be propagated through multiple generations despite negative effects on the organism's health (Fan et al., 2008; Liau et al., 2007). The effects of mutations in multiple protein-coding genes, rDNA genes, and tRNAs as well as their additive biological effects are currently unknown and additional studies are needed to fully understand the implications of these differences on fitness at individual and population levels.

Whereas novel combinations of nuclear alleles have been widely cited as the most likely cause of hybrid vigor (Arnold, 1997; Barton, 2001; Stebbins, 1959), reduced fitness or hybrid breakdown in interspecies hybrids may be explained by disruptions in mitochondrial function due to incompatibilities between the nuclear and mitochondrial genomes (Burton et al., 2006). These effects have been noted in an array of organisms including arthropods, plants, yeast, mice, insects, and birds (Ellison and Burton, 2008; Johansen-Morris et al., 2006; Lee et al., 2008; Nagao et al., 1998; Sackton et al., 2003; Tieleman et al., 2009). Bison–cattle hybrids are an excellent mammalian model to study these issues, since the large number of observed sequence differences — many of which presumably result in functional changes — likely affect nuclear–mitochondrial gene interactions and may also affect fitness.

The data generated in this study is also valuable in understanding the taxonomic classification of American bison, which has been

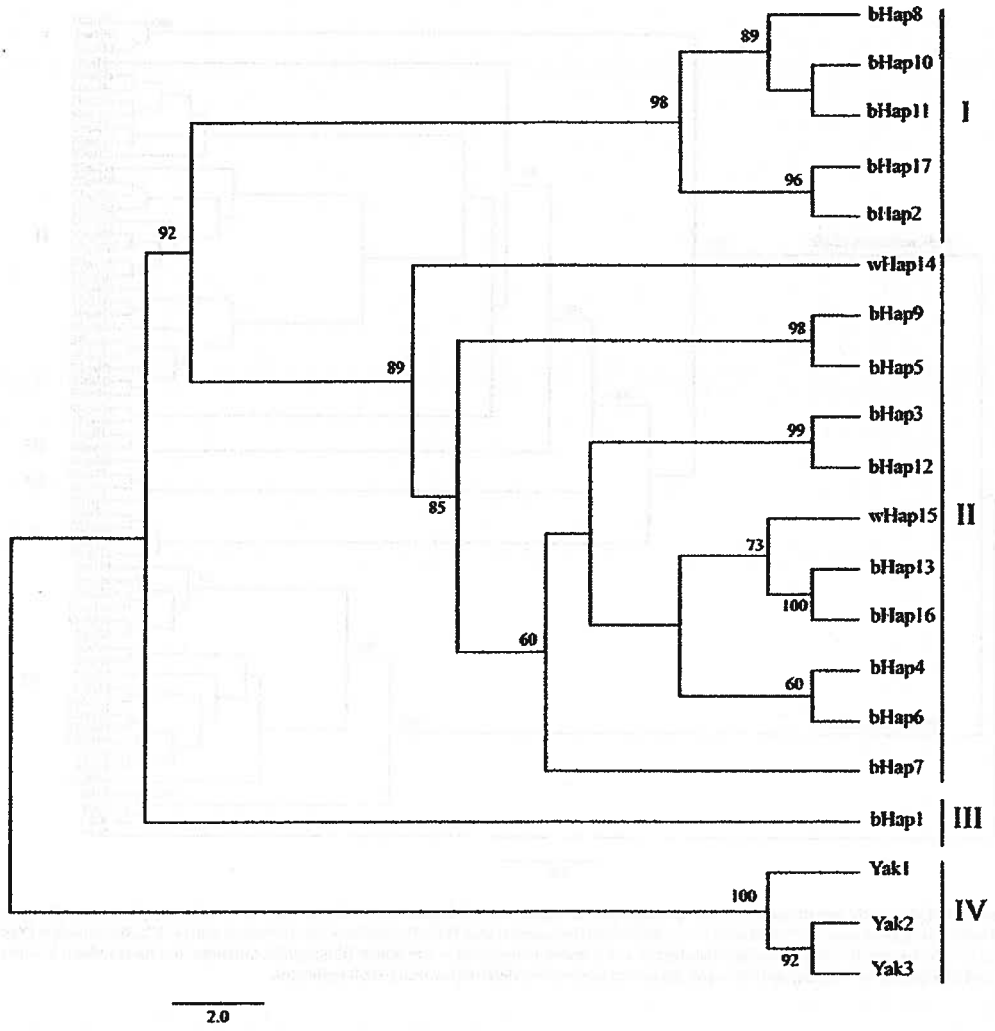

Fig. 4. Maximum likelihood phylogenetic tree of bison complete mtDNA haplotypes rooted by *Bos grunniens* (yak). Clades: I — plains bison (Fort Niobrara NWR, Yellowstone NP, National Bison Range, private herd); II — plains and wood bison (Texas State Bison Herd, private herd, Elk Island NP); III — previously published bison sequence (Achilli et al., 2008); IV — Yak. NP, National Park; NWR, National Wildlife Refuge. Bootstrap values generated by sampling 1000 replicates.

debated at the genus, species, and subspecies level. The paraphyletic nature of the *Bos* and *Bison* lineages has been shown based on both nuclear polymorphisms and analysis of individual mitochondrial regions (Burzynska et al., 1999; Decker et al., 2009; Janecek et al., 1996; Miyamoto et al., 1989; Wall et al., 1992). We also identified paraphyly of the *Bos* genus with respect to *Bison* based on whole mtDNA genome analysis, with bison and *Bos grunniens* forming a distinct clade from *Bos taurus* and *Bos indicus* (Fig. 3). Our data do not support the genus designation of *Bison*. The recent accumulation of molecular data, together with the fact that members of *Bison* can produce viable offspring with several species of *Bos* (van Gelder, 1977), indicate that the *Bison* and *Bos* genera should be reunited.

We identified significant phylogenetic substructure among bison (Fig. 4), which can be used to assess the relationship between the currently recognized subspecies of American bison (wood and plains). The two wood bison haplotypes do not form a single clade (wHap 14, 15) and are mixed with plains bison haplotypes (Figs. 1 and 4). The fact that both of these haplotypes fall into a clade with plains bison suggests that wood bison may never have been a genetically distinct subspecies, although it is also possible that one or both of these sequences are derived from the introduction of plains bison into wood bison herds in the 1920s (Banfield and Novakowski, 1960; Roe, 1970). Regardless of the source of these haplotypes, however, current populations of *B. bison bison* and *B. bison athabascae* are not significantly different with respect to their mitochondrial genomic sequences and should not be considered subspecies. It does appear, however, that the currently listed *B. bison athabascae* are an important source of genetic diversity for the species, since the two wood bison haplotypes were not identified in any of the plains bison populations (also see Wilson and Strobeck, 1999).

Haplotype analysis reveals further insights into the history and population structure of the bison species. Given the severe bottleneck experienced by bison in the late 19th century, when the total number of individuals in the species declined from approximately 30 million (Flores, 1991; McHugh, 1972) to less than 1000 (Coder, 1975; Soper, 1941), it was somewhat unexpected that 16 bison mtDNA haplotypes were identified in this study (Supplemental Materials Table 1; Fig. 1). Even excluding the haplotypes which differ by only 1–2 nucleotides (e.g., bHap12/bHap3), at least 10 distinct bison mtDNA types were identified in this study. Others have also identified high levels of genetic diversity in the bison nuclear genome (Halbert and Derr, 2008; Wilson and Strobeck, 1999), which may be due to the wide-

spread distribution of bison prior to and following the bottleneck, a short bottleneck length, and rapid population expansion following the bottleneck.

In addition, the relationships among bison haplotypes (Fig. 1) are generally reflective of the historical records of population establishment and genetic distances based on nuclear data (Halbert and Derr, 2008). For example, the close relationship of haplotypes identified at the National Bison Range (bHap2) and Yellowstone National Park (bHap17) is explained by the shared history of the herds (Halbert and Derr, 2007) and is also reflected in the nuclear genome (Halbert and Derr, 2008). Additionally, we found that the private bison herd sampled in this study harbors both unique haplotypes and haplotypes representative of several sources including Fort Niobrara National Wildlife Refuge (cHap10), the National Bison Range (cHap2), and the Texas State Bison Herd (cHap13) (Supplemental Materials Table 1). This finding is not surprising given the fact that, like many private herds, this herd was founded with bison from many sources.

5. Conclusion

By using modern sequencing technologies to obtain whole mtDNA genome sequences from several bison and bison–cattle hybrids in this study, we have examined the potential effects of hybridization between American bison and cattle, analyzed the phylogenetic relationship between *Bison* and *Bos*, constructed the first whole mtDNA phylogenetic tree of American bison, and identified population substructure and subspecific relationships among bison populations. Additional studies are now clearly needed to understand the effects of cattle mtDNA in bison on mitochondrial function and physiology, evaluate the potential for fitness differences due to cattle mtDNA in bison, and define the taxonomic relationship of European bison (*Bison bonasus*, the only other extant species in the *Bison* genus) to American bison and the *Bos* genus.

Supplementary materials related to this article can be found online at doi:10.1016/j.mito.2010.09.005.

Acknowledgements

The authors thank Dr. Nicole Ramlachan for assistance with DNA extractions.

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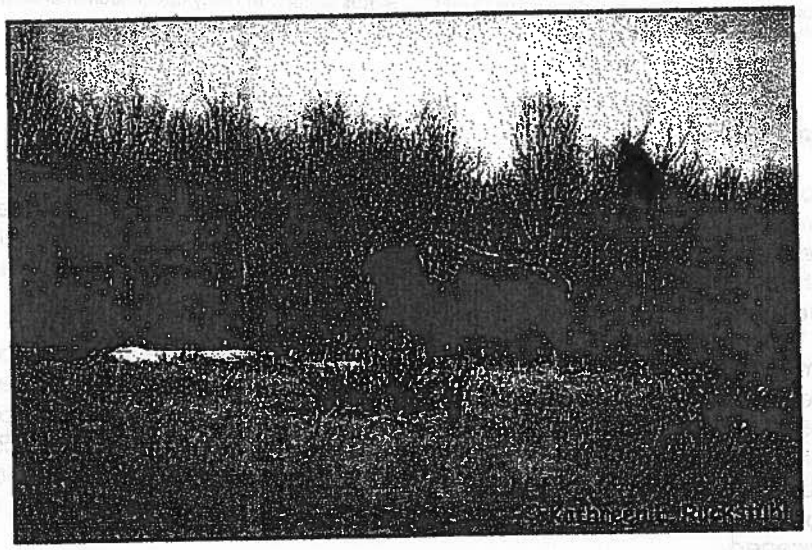
Species Profile

Wood Bison

Scientific Name: *Bison bison athabascae*
Taxonomy Group: Mammals
Range: Yukon, Northwest Territories, British Columbia, Alberta
Last COSEWIC Assessment: May 2000
Last COSEWIC Designation: Threatened
SARA Status: Schedule 1, Threatened

Go to advanced search

Quick Links: | Photo | Description | Distribution and Population | Habitat | Biology | Threats | Protection | Recovery Initiatives | Recovery Team | National Recovery Program | Documents



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Description
The Wood Bison is the largest Canadian terrestrial mammal. It is dark brown, with a massive head, a high hump on its large shoulders, and long shaggy hair on its shoulders and front legs. The short legs end in rounded hooves. The short and black horns curve inward on the males, but are straight on the females. There are two moults every year, one in the spring and one in the fall. The males are larger than the females; an adult male measures 3.04 to 3.8 m in length and 1.67 to 1.82 m in height (at the shoulders), and weighs between 350 and 1000 kg. Wood Bison are generally taller and less stocky than Plains Bison. Both Wood and Plains Bison are considered by some to be subspecies of the American Bison, but their actual systematic status is unclear and controversial.

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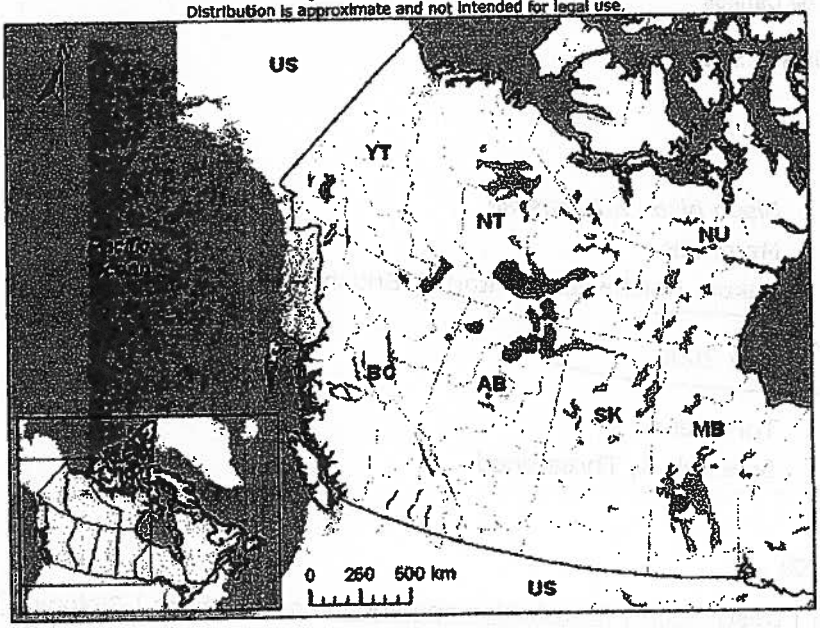
Canadian Distribution of the Wood Bison (shown in red) [1,2]

Distribution is approximate and not intended for legal use.



[1]Author: Canadian Wildlife Service, 2004

[2]Data Sources: The main source of information and data is the COSEWIC Status Report. In many cases additional data sources were used; a complete list will be available in the future.

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Distribution and Population

The Wood Bison, considered a northern subspecies of the American Bison, is a Canadian endemic (occurs only in Canada). In the past, it was found in northeastern British Columbia, northern Alberta, northwestern Saskatchewan, Yukon, and southwestern Northwest Territories. Today, there are herds of wood bison in Alberta, Manitoba, British Columbia, Yukon, and southwestern Northwest Territories.

A recovery program established in 1957 has aided the population of Wood Bison to increase from 200 in 1957, to over 3,000 free-roaming bison in 1999. Historical estimates suggest that there once were over 168,000 Wood Bison in Canada. The latest population estimates count 3,536 bison, with 2,828 in the wild and 708 in captivity, free of brucellosis and tuberculosis. There are six populations in the wild and four captive breeding herds. Two wild herds exceed the minimum viable population of 400 individuals. In 2000, the species was re-examined using new criteria and reaffirmed as threatened.

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Habitat

Wood Bison are found in the open boreal and aspen forests where there are large wet meadows and slight depressions caused by ancient lakes. The population in the Mackenzie Bison Sanctuary (NWT) uses wet meadows and willow savannas in summer and winter and forests in the fall.

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Biology

The Wood Bison is a long-lived species, living up to 40 years. Both males and females reach sexual maturity at 2-4 years of age, but males usually mate at about 6 years of age or older -- when they can compete with larger bulls for females. The rut is in August and early September. After a gestation period of 270 to 300 days, cows give birth to a single red calf in May; twins are rare. Females usually give birth twice in three years. Wood Bison feed mainly on sedges and grasses,

but also on the leaves and bark of trees and shrubs (primarily willow) and lichens. The wolf is the bison's main predator (other than humans), but newborn calves can be taken by bears.

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Threats
Disease (anthrax, brucellosis, and tuberculosis), cross-breeding with Plains Bison, and habitat loss through human development, agriculture, and forestry and petroleum resource development are the main threats faced by Wood Bison. Anthrax is a fatal disease for herbivores, that is contracted through bacteria found where the ground has been contaminated by infected carcasses. Anthrax was a major cause of death for the Wood Bison before 1978 and could easily recur, especially in particularly wet years. The Wood Bison in Wood Buffalo National Park were infected with bovine tuberculosis and brucellosis (and began hybridizing) when Plains Bison were moved to the park from 1925-1928. The Plains Bison had contracted the two diseases from domestic cattle with which they had been held, and during cross-breeding experiments. Wood Bison can drown during spring floods or when they venture onto thin ice; in 1961 and 1974 several hundred bison drowned in a flood. These events likely do not affect bison populations over the long term. However, the periodic floods supplied water to elevated ponds and wet meadows where the bison feed. With the construction of the W.A.C. Bennett Dam, flooding has been largely controlled and the meadows and ponds have dried. As a result, the vegetation has converted from sedges, which provided important winter forage, to grasses and shrubs, which are thought to be less suitable as food. The conversion of lands for agriculture or other development has limited the habitat of the Wood Bison. Biting insects are a problem for the herds in captivity, since their movements are restricted by fences and they thus cannot escape the insects; this has resulted in reproduction problems and mortality. Also, so-called pure blood Wood Bison are kept apart from Plains Bison and bison hybrids to protect the genetic integrity of the subspecies. This limits the habitat which can be used to reintroduce the Wood Bison, since part of the habitat is occupied by other bison.

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Protection

Federal Protection

The Wood Bison is protected under the federal *Species at Risk Act* (SARA). More information about SARA, including how it protects individual species, is available in the *Species at Risk Act*: A Guide.

A law to protect the Wood Bison from hunting was first introduced in 1877. In the Northwest Territories, the Yukon, British Columbia, and Alberta, the species is protected against such activities as capture, harassment, trade, and killing. The species is fully protected in the Mackenzie Bison Sanctuary in the Northwest Territories, and only a limited number of tags for hunting bison are available annually. National parks, such as Wood Buffalo National Park, ensure some protection for the bison. Regulations and cooperative agreements with native peoples protect the Wood Bison against hunting when it is reintroduced into an area.

Provincial and Territorial Protection

To know if this species is protected by provincial or territorial laws, consult the provinces and territories websites.

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Recovery Initiatives

Status of Recovery Planning

Recovery Strategies :

Name National Recovery Plan for the Wood Bison (Bison bison athabascae)
Status Revision received by leads
Number of Action Plans 0

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Recovery Team

Bison Recovery Team

Cormack Gates - Chair - University or college
Phone: 403-220-6605 Fax: 403-284-4399 Send Email

Hal Reynolds - Chair - Environment Canada
Phone: 780-951-8702 Fax: 780-495-2615 Send Email

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Recovery Progress and Activities

Summary of Progress to Date

Historically Wood Bison ranged throughout the boreal forest of northern Alberta, northeastern British Columbia, northwestern Saskatchewan, the southwestern Northwest Territories, and much of Yukon and Alaska. In the early 1800s, Wood Bison numbers were estimated at 168,000 animals, but by the late 1800s only a few hundred animals remained. In 1978, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) assessed Wood Bison as Endangered. As a result of an active recovery program, Wood Bison were reassessed as Threatened by COSEWIC in 2000. Since then numbers have continued to increase. As of 2006, there were an estimated 4188 Wood Bison in seven free-ranging, disease-free herds, 6216 animals in four diseased, free-ranging herds, and 1029 animals in captive conservation (public and private) and research herds.

Summary of Research/Monitoring Activities

The Wood Bison Reproduction Research Group was established in 2006 to further collaboration among the University of Saskatchewan, University of Calgary, Calgary Zoo, Government of the Northwest Territories, and the Parks Canada Agency. The objective of the research program is to characterize and understand the reproductive cycle in bison and to further develop reproductive technologies for the conservation of valuable genetic material from diseased wood bison populations. Three male and 15 female wood bison are being raised in captivity at the Western College of Veterinary Medicine, University of Saskatchewan, as part of these studies.

Other studies are underway to address the management of genetic diversity for captive and free-ranging bison herds.

Summary of Recovery Activities

The Government of the Northwest Territories has initiated an enhanced disease surveillance program for the Mackenzie and Nahanni herds to confirm the disease-free status established through previous ongoing testing efforts.

In March 2006, the Hook Lake Wood Bison Recovery Project was terminated and the herd was destroyed after Tuberculosis was detected in one of the founder animals and several captive born bison.

In various locations, captive herds are being maintained to provide stock for reintroductions, free-ranging populations are being protected, and the growth of small, disease-free, populations is being promoted.

In April 2006, 30 surplus wood bison calves were transferred from Elk Island National Park to Lenski Stolby Nature Park near Yakutsk, Sahka Republic (Yakutia), Russia. This project was supported by the Recovery Team on the basis of contributing to the global security of wood bison. It was considered an additional opportunity to secure survival of the subspecies within a geographically separate population.

URLs

Parks Canada: Elk Island National Park of Canada:
www.pc.gc.ca/pn-np/ab/elkisland/natcul/natcul1bil i_E.asp

Hinterland Who's Who: North American Bison:
www.hww.c a/hww2.asp?cid=8&id=97

Yukon Wood Bison:
http://www.yfwmb.yk.ca/comanagement/mgmtplans /bisonplan/yukon.html

Wildlife at Risk in BC: Wood Bison:
ht tp://www.env.gov.bc.ca/wld/documents/wbison.pdf

Hinterland Who's Who: North American Bison: http://www.hww.ca/hww2.asp?pid=1&cid=8&id=97

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Documents

Permits and Related Agreements (7 record(s) found.)
7 record(s) found.

Permits and Related Agreements

Explanation for issuing permit(#06-01-17529), persuant to the provisions of section 73 of SARA (2006)

Thirty wood bison calves from the National Wood Bison Conservation Herd at Elk Island National Park (EINP) will be translocated to the Republic of Sakha, Russian Federation. These bison will be released to the wild after a period of acclimatization a...

Explanation for issuing permit(#06-01-17530), persuant to the provisions of section 73 of SARA (2006)

Twenty-five wood bison calves from the National Wood Bison Conservation Herd at Elk Island National Park (EINP) will be translocated to the Western College of Veterinary Medicine, University of Saskatoon for research on assisted reproductive technolo...

Explanation for issuing permit(#10-01-56818), persuant to the provisions of section 73 of SARA (2010)

Thirty wood bison calves from the National Wood Bison Conservation Herd at Elk Island National Park (EINP) will be translocated to the Republic of Sakha, Russian Federation. These bison will be released to the wild after a period of acclimatization a...

Explanation for issuing permit(#7), persuant to the provisions of section 73 of SARA (2006)

Transfer (possession) of Wood Bison at the Edmonton International Airport. This is an international cooperative conservation and recovery project between the Government of the Republic of Sakha (Yakutia) and Environment Canada, whereby 30 wood bison...

Explanation for issuing permit(#EINP 2008-01), persuant to the provisions of section 73 of SARA (2008)

Elk Island National Park is a fenced environment preventing both the dispersal and predation of large mammals. Large mammal populations, including wood bison, must be actively managed within defined carrying capacity limits in order to maintain popu...

Explanation for issuing permit(#WB-2008-1719), persuant to the provisions of section 74 of SARA (2008)

An aerial reconnaissance will be carried out using a fixed wing to locate large mixed groups of bison and this will be followed up with a ground-based count. The ground-based count will be facilitated by rotary wing aircraft to drop off the research...

Explanation for issuing permit(#WB08-1006), persuant to the provisions of section 74 of SARA (2009)

The BBC is proposing to film Bison/wolf interactions in Wood Buffalo National Park for a new series, 'Our Frozen Planet'. The project is scheduled to begin in late February 2009 and end in Late March 2009. The filming will take place when snow is s...

Date Modified: 10/07/2010

Environment Canada Environnement Canada

Canada

Wood-plains bison hybridization and the loss of genetic diversity in small herds

March 24, 2011

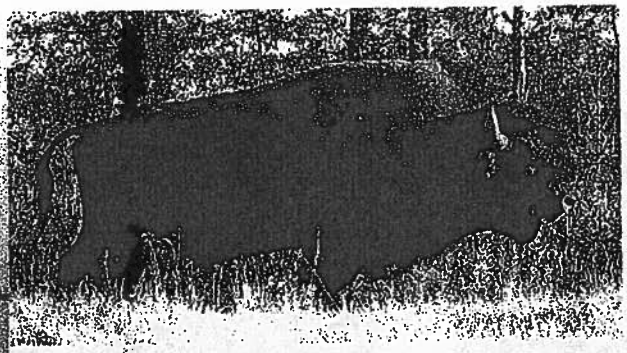
Greg Wilson
Environment Canada
Canadian Wildlife Service

April 28, 2011

Outline

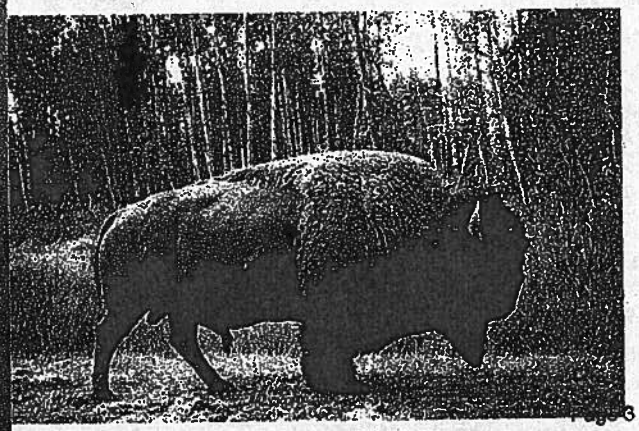
- History of wood bison in Canada
- Subspecific hybridization
- Loss of diversity in a recently founded population
- Hybridization with cattle

Wood and plains bison



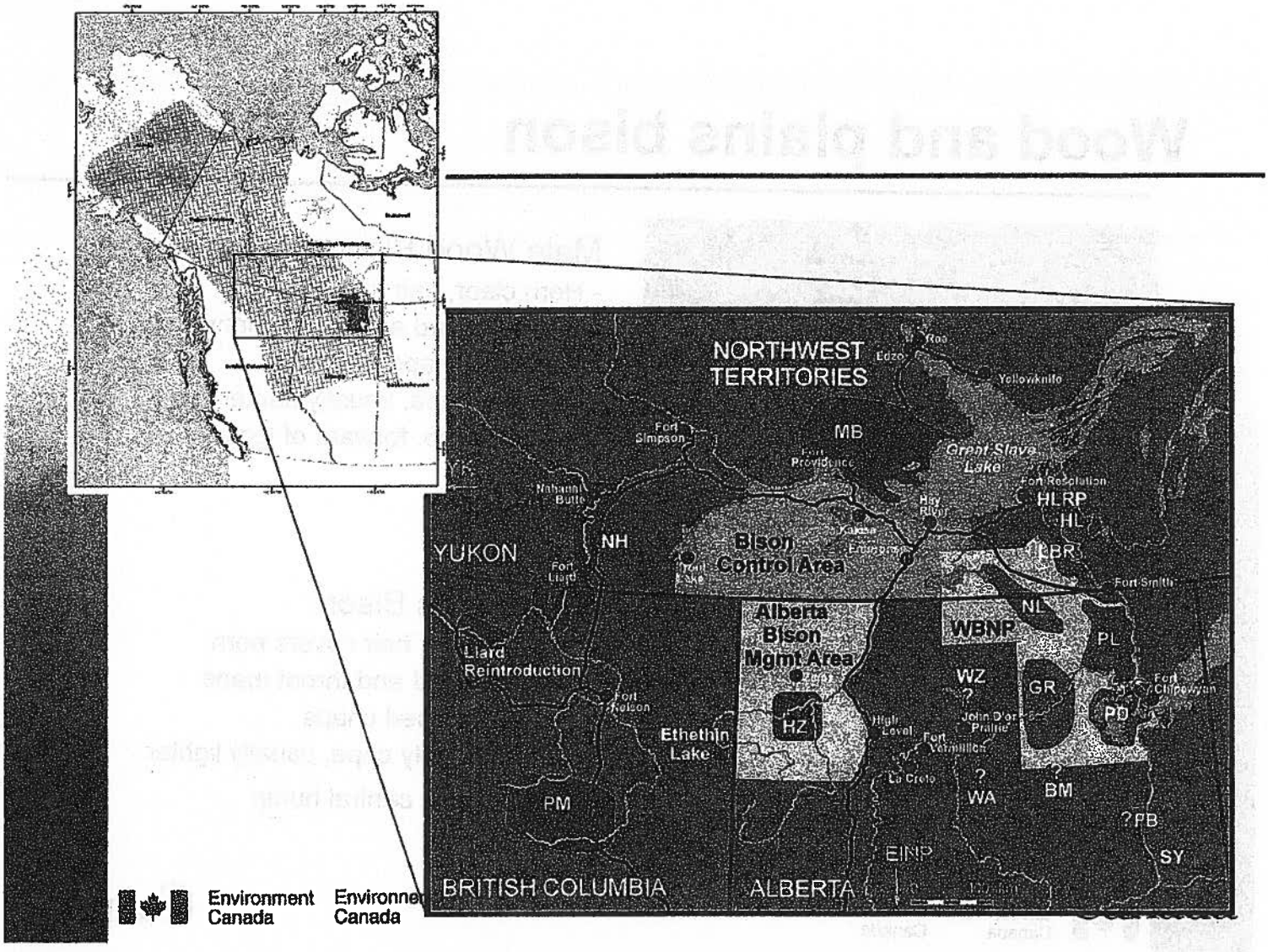
Male Wood Bison

- Horn clear, hair shorter
- Shorter beard and throat mane
- Reduced chaps
- No clear cape, usually darker
- Larger hump, forward of leg axis



Male Plains Bison

- Thick, woolly hair covers horn
- Longer beard and throat mane
- Well-developed chaps
- Distinct woolly cape, usually lighter
- Lower, more central hump


NORTHWEST TERRITORIES
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Yellowknife
Fort Simpson
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Fort Providence
Great Slave Lake
Fort Resolution
Nahanni Butte
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Hay River
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HL
YUKON
NH
Bison Control Area
Kakisa
Enterprise
Fort Liard
Trout Lake
LBR
Fort Smith
Alberta Bison Mgmt Area
NL
WBNP
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Liard Reintroduction
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GR
Fort Chipewyan
Fort Nelson
PD
HZ
High Level
John D'or Prairie
Ethelbin Lake
Fort Vermilion
La Crete
?
WA
BM
PM
?PB
EINP
SY
BRITISH COLUMBIA
ALBERTA
Environment Canada
Environnement Canada

History of Canadian wood bison

Prior to 1800: ~100,000 wood bison existed mostly in northern Alberta, NWT and Alaska

1896-1900: Numbers reached a low of about 250

1877-1911: Various measures undertaken to save wood bison from extinction

1922: Wood Buffalo National Park established to protect 1500 remaining wood bison

Plains bison in Canada

- Banff NP
  - In 1897, 3 bison from Goodnight, 13 from Lord Strathcona (Alloway / McKay lineage)

- Elk Island National Park (EINPP)
  - Established in 1907 from 410 Pablo/Allard animals
  - Some animals transferred between Banff and EINPP
  - All but ~50 EINPP bison shipped to Buffalo National Park
  - More Banff, Pablo/Allard and some Corbin (Buffalo Jones) bison shipped to Buffalo National Park

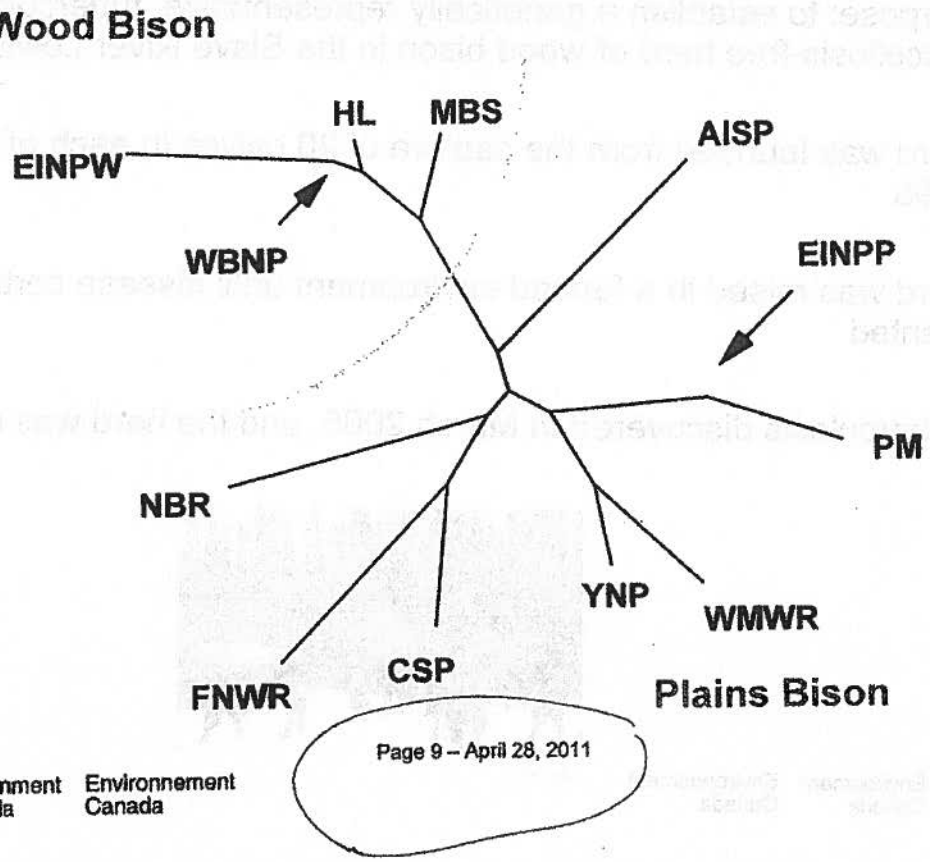
Buffalo National Park

- Cross-breeding experiments between plains bison and cattle were undertaken
  - Deemed to be unsuccessful
- Bison found to be infected with tuberculosis
  - Brucellosis later discovered
- 6673 animals shipped to WBNP in 1925
- Wood, plains bison hybridized, both diseases spread

History of wood bison cont'd

- In 1959, an isolated herd of wood bison were found in WBNP
- 18 were shipped to Mackenzie Bison Sanctuary in 1963, and 16 to EINPW in 1965
- These herds were later found not to be pure
  - Morphological and genetic evidence of hybridization

Genetic relationships between wood and plains bison



The Hook Lake Wood Bison Recovery Project

- Purpose: to establish a genetically representative, tuberculosis- and brucellosis-free herd of wood bison in the Slave River Lowlands
- Herd was founded from the capture of 20 calves in each of 1996, 1997, and 1998
- Herd was raised in a fenced environment until disease certification could be granted
- Tuberculosis discovered in March 2005, and the herd was depopulated

Genetic diversity in the HLWBRP

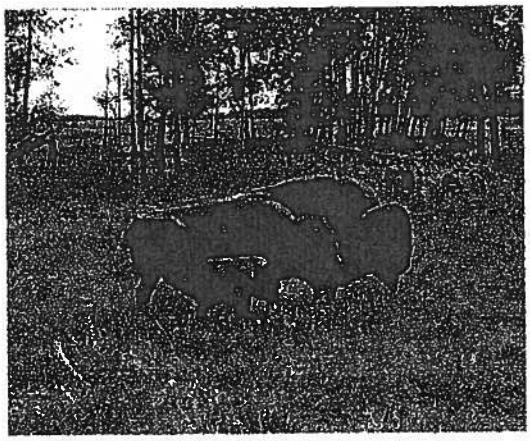
Population	Size	# Alleles	Exp Het	1/pI
WBNP	~4500	6.55	0.552	57 000 000
HLWBRP	58	5.55	0.508	5 600 000

Genetic diversity in the HLWBRP

Population	Size	# Alleles	Exp Het	1/pI
WBNP	~4500	6.55	0.552	57 000 000
HLWBRP	58	5.55	0.508	5 600 000
1999 calves	9	3.36	0.490	3 700 000
2000 calves	24	4.36	0.423	250 000
2001 calves	25	4.36	0.484	1 700 000
2002 calves	25	4.64	0.455	833 000
Total calves	**83**	**5.18**	**0.459**	**740 000**

Differential reproductive success

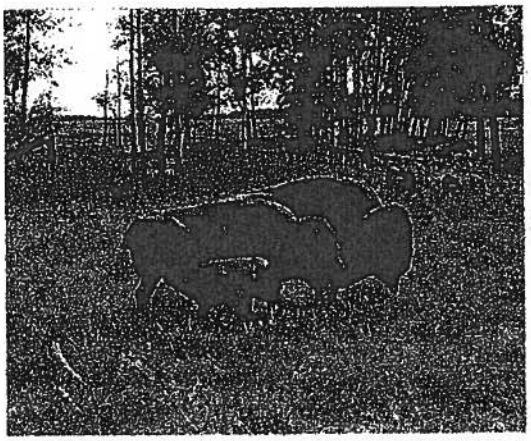
Sire ID	Total	% Total
31	1	1.2
37	30	36.1
40	1	1.2
44	23	27.7
48	5	6.0
B5	1	1.2
B11	8	9.6
B13	13	15.7
O5	1	1.2



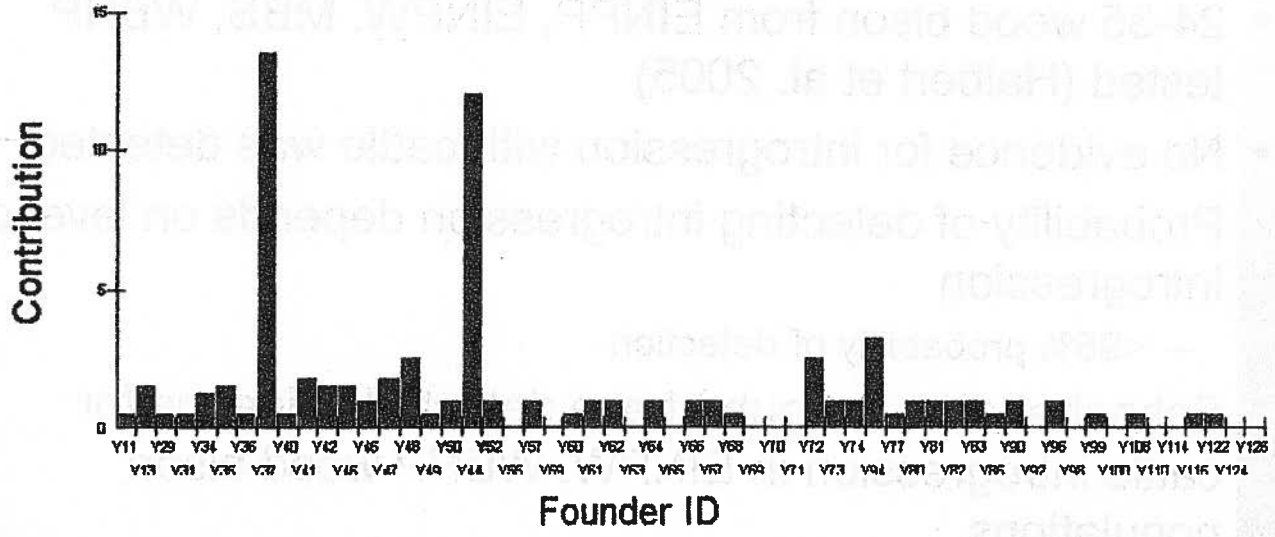
Differential reproductive success

Sire ID	Total	% Total
31	1	1.2
37	30	36.1
40	1	1.2
44	23	27.7
48	5	6.0
B5	1	1.2
B11	8	9.6
B13	13	15.7
O5	1	1.2

B5, B13, and O5 are sons of 37, while B11 is the son of 44



Founder Contributions



Cattle hybridization

- 24-35 wood bison from EINPP, EINPW, MBS, WBNP tested (Halbert et al. 2005)
- No evidence for introgression with cattle was detected
- Probability of detecting introgression depends on level of introgression
  - <95% probability of detection
- Schnabel et al. (unpub.) have detected evidence for cattle introgression in EINPW, WBNP wood bison populations
  - Absence of introgression in EINP plains bison suggests hybridization occurred in Buffalo National Park population

Summary

- WBNP quite genetically variable
  - Continued gene flow from this population would increase diversity in other herds, reduce loss through time
  - Complicated by disease issue

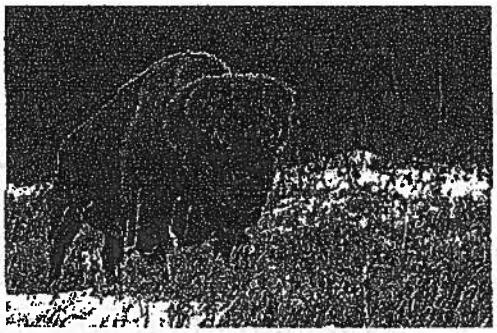
Unlikely pure wood bison exist

Must be aware of capturing and keeping genetic diversity through time

Elk Island National Park

Government of Canada to Send Wood Bison to Russian Conservation Project

Environment Canada (Canadian Wildlife Service) and Parks Canada Agency have developed an agreement with the Republic of Sakha (Yakutia) in Russia, to transfer approximately 30 wood bison from Elk Island National Park to the Republic of Sakha. Proposed timing for the transfer from Elk Island to Sakha is March 14-18, 2011. Read the Press Release



Park Canada contributes to survival of wood bison, a Species at Risk
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This will be the second transfer to Russia. In 2006, 30 wood bison were transferred to Lenskie Stolby Nature Park in the Republic of Sakha. This transfer was successful and the herd has reproduced and increased. Establishing a herd of wood bison in the Republic of Sakha fulfills a national aspiration to *rebuild an* ecosystem analogous to the one that existed in the area before the extinction of steppe bison. The renewal of a population of large herbivores will augment the natural capital and biodiversity of the region.

In Canada, the federal government is responsible for the recovery of listed species, through the Species at Risk Act. Currently, there are an estimated 11,000 wood bison in Canada. Wood bison (*Bison bison athabascae*) are listed as a threatened species: "a species likely to become endangered if the limiting factors are not reversed" in Schedule 1 of the *Species at Risk Act* (SARA). The Canadian Wildlife Service will develop a strategy including recommendations to create the best possible future for wood bison. Parks Canada supports this work by maintaining the wood bison herd in Elk Island National Park, and will participate in the implementation of the Recovery Strategy.

The wood bison herd has been in Elk Island National Park (EINP) since 1965. Serving as a recovery herd, the wood bison offer a source of disease-free and genetically pure animals for reintroduction projects nationally and internationally. The wood bison living in Elk Island National Park are not native to the area. Parks Canada maintains the wood bison herd in Elk Island National Park to serve as a nursery herd to establish and augment wood bison populations in Canada. This includes managing the population and providing surplus animals to conservation initiatives such as the transfer to the Republic of Sakha (Yakutia), on the basis of contributing to the global security of wood bison. In 2001, this project was included in the draft *National Wood Bison Recovery Plan* as an opportunity to secure survival of the subspecies in a geographically separate population. Environment Canada, through its branch the Canadian Wildlife Service, is the lead on the Wood Bison Recovery Strategy and the lead on the transfer. The Canadian Food Inspection Agency veterinarians conduct the disease testing and ensure animal health. Parks Canada hosts and manages the herd of wood bison at Elk Island National Park.

The Government of Canada and the Republic of Sakha (Yakutia) are taking all possible measures to ensure a safe transfer for the animals. The Canadian Food Inspection Agency in cooperation with the Republic of Sakha (Yakutia) has developed a disease testing and treatment protocol for the animals and a Veterinary Health Certificate that both countries have agreed to for this export-import of wood bison.

Parks Canada has a proven track record of effective recovery for species at risk in national parks. Contributing to the survival of species at risk has been an important role historically for Elk Island National Park for more than a century.

Elk Island has played a key role in the conservation of both plains bison and wood bison since 1907. Some of the world's last plains bison were brought to the park and the species began its recovery from the brink of extinction. Elk Island National Park has made lasting contributions to wildlife conservation through its expertise and management (e.g. wood bison, plains bison, elk, trumpeter swans) for over a century. In Canada, relocations have been made in British Columbia, Alberta, Saskatchewan, Ontario and the Yukon Territory. Internationally, the Park has supported the relocation of elk to Kentucky, Tennessee and North Carolina; in 2006, 30 wood bison were sent to Russia, and in 2008, 54 were transferred to Alaska. During the park's history, Elk Island has successfully provided a total of 855 wood bison, 1014 plains bison, and 4633 elk to conservation initiatives benefiting the species.

For more information on the draft *National Wood Bison Recovery Plan* or the *Species at Risk Act*, please visit the Species at Risk Registry.

Date Modified 2012-01-23

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